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THE TENTH NATIONAL CONFERENCE ON WHEAT UTILIZATION RESEARCH was held November 16-18, 1977, in Tucson, Arizona. The objective of the biennial conference is to provide a forum for exchanging information and ideas as well as for discussing problems related to production, handling processing, and merchandising of wheat.

Sponsors of the Conference were Agricultural Research Service, U.S. Department of Agriculture; Great Plains Wheat, Inc., and affiliated State agencies; Millers' National Federation; National Association of Wheat Growers; and Western Wheat Associates, USA, Inc., and affiliated State agencies. Chairman of the Program Committee was Dr. Robin M. Saunders, Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Albany, California.

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THE WHEAT SITUATION IN ARIZONA

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Small grain production in Arizona has been directed largely toward barley until the last few years because feeder operations and dairies were the principal markets. Production in the traditional wheat states has supplied the milling industry and other large markets while the small Arizona wheat acreage had little impact on the wheat market until the last three years.

In 1965, a group of foreign wheats were compared for potential yield and lines from the SIMMIT research program in Mexico dramatically outyielded Ramona 50, the principal variety grown at that time. After two years of comparisons, growers recognized that the new semi-dwarf wheat yields exceeded both wheat and barley varieties in local use and that cultural management was easier since lodging was a small hazard. Although cattle feeding operations resisted wheat as a feed, they began to use some of this grain while paying a premium for barley. Meanwhile, the local Arizona milling company had begun to use two of these new varieties in blends with imported hard red winter wheat.

As feeders gained experience in rolling wheat and university feeding trials revealed the desirability of new varieties, feeding operations increased purchases. These changes accelerated acceptance so that semi-dwarf wheat acreage increased nine-fold from 1965 to 1974 with most of this production being used as feed. At this point wheat constituted 74 percent of small grain acreage in contrast to 1965 when wheat made up only 14½ percent of the total plantings.

In the fall of 1974, exporters offered advance contracts for milling wheats, such as Cajeme and INIA 66, and wheat acreage increased another 36 percent to 320,000 acres for 1975 harvest. This was followed by unexpected contracting for durum wheat during the summer and fall of 1975, and Arizona farmers increased wheat acreage an additional 35 percent for harvest in 1976. Only 30,000 acres of durum wheat had been grown in the 1974-75 season, but 319,000 acres were reported in 1976, and this represented 74 percent of the wheat crop.

The rapid rise in wheat acreage was gratifying to farmers since yields were over 50 percent higher than 1965 production and advance contract prices for export were much more than feed prices. Milling contracts in 1975 were approximately $6.00 per cwt. while 1975-76 durum contracts ranged from $5.75 to $7.35. However, many growers had little experience in growing durum wheat and dockage penalties for "yellow berries" were disconcerting to individuals who were encountering relatively large discounts for the first time.

WHEAT ACREAGE AND YIELD - ARIZONA

Year	Acres	Yield Per Acre	Percent of Small Grain Acreage	Marketing Season
	1000 Acres	Lbs.	Percent	Dollars/Ton
1965	26	2700	14.5	52.00
1970	150	4140	51.7	47.00
1971	173	4080	59.2	57.00
1972	170	4020	60.9	54.70
1973	216	4200	64.4	86.70
1974	235	3960	70.1	104.60
1975	320	4260	74.6	105.00
1976	431	4500	81.2	131.00

Quality of the 1976 durum wheat crop was quite favorable, however, and many experienced farmers marketed a high percentage of their crop as Hard Amber Durum. Random sampling of 300 truckloads graded 69.5% Hard Amber Durum, 14% Amber Durum, and 16.5% Durum, whereas random sampling of 100 carlots graded 84% Hard Amber Durum, 9% Amber Durum, and 7% Durum.

The improved quality in railway car loadings undoubtedly reflected the effects of blending qualities since two very large producers marketed high proportions of their crops as Hard Amber Durum wheat. Both used blending methods and one of these, with several thousand acres, was able to ship over 98% of the crop as Hard Amber Durum even though some on-farm truckloads contained high percentages of "yellow berries."

The variety Produra, which had made up most of the 1975 durum wheat production, caused few "yellow berry" problems, but Cocorit was much more subject to this problem under excessive irrigation or deficient nitrogen supplies during the seed-forming stages in 1976. Approximately 19 million pounds of Cocorit seed were brought in from Mexico through four ports for use in Arizona and California to plant a majority of Arizona wheat acreage.

Quality of the milling varieties Cajeme and INIA 66R have been found very acceptable for Arizona flour uses and relatively little wheat is imported for blending of varieties. These do not require special grinding and store better because of low moisture content. These varieties are good for family flour and make excellent flour for tortilla shops that make up a large market.

Arizona advantages in growing wheat are high yields, relatively stable yield and quality, low smut incidence, few disease problems, low uniform moisture content, earlier harvest than the midwest, and proximity to west coast seaports. Disadvantages include the lack of bread wheat varieties and a reputation for having lower quality durum varieties available. Exclusion from the government loan premium of 7.5 cents per bushel for durum wheat seems detrimental, but exporters state that Arizona durum in 1978 will be shipped to Italy, a country which is reported to have discriminating taste for spaghetti quality.

Low moisture content in grain grown in Arizona amounts to about five percent less moisture for the purchaser to pay for and the consistency of moisture content is an advantage to the milling company. On the other hand, the water requirement of 25 acre-inches to raise this crop is much less per unit of grain produced than water use for a grain sorghum crop grown in the summer months. This fact makes wheat fit into the farmer's irrigation calendar in an opportune way.

The cost of growing wheat in Arizona is high so that present contract prices of $5.00 per cwt. for durum wheat are not encouraging large plantings. Some estimates forecast 30 to 35 percent of the 1976 acreage and much of this will be in Yuma County where water costs are lower. However, if market price increases to the 1976 level in the future, Arizona farmers will be very interested in large acreages again.

WHEAT GRADING SYSTEMS OF THE MAJOR WHEAT EXPORTING COUNTRIES: ARGENTINA, AUSTRALIA, CANADA, FRANCE, AND U.S.A.

by

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INTRODUCTION

Before proceeding into an examination of the wheat grading systems of five major wheat exporting countries, it is important to understand why they have grading systems. The systems were designed and adopted in order to enable the countries to establish the market value of their grains. Foreign buyers rely on grades to obtain consistent quality from shipment to shipment. Accordingly, an efficient and reliable grading system is essential to the effective marketing of wheat.

In establishing an effective grading system, the first step is usually to differentiate between different classes of wheat such as hard red spring, hard red winter, soft white spring, amber durum, and so on. Each class can then be subdivided into subclasses or grades, usually on the basis of physical characteristics such as test weight and appearance, and presence of other grains and foreign matter. Physical characteristics which are assessed visually are generally used in most countries that have wheat grading systems to make the grading operation as practical as possible. Limits in moisture content are usually adopted for the standard or straight grades. Grades or subclasses can be further subdivided according to other physical, and in some cases, chemical criteria in order to more precisely define their market value. A very important chemical criterion used to subgrade certain classes of bread wheat is protein content. Australia, Canada, France, and the U.S.A. have adopted systems for the segregation of bread wheat on the basis of protein content and apply this segregation to their respective grading systems.

Let us now look at the grading systems of the five major wheat exporting countries, Argentina, Australia, Canada, France, and the U.S.A.

ARGENTINA

In normal years about 50% of the wheat produced in Argentina is exported and during the 10-year period 1960-1970 provided some 15% of total Argentine export earnings. Their particular markets have been throughout South America and Western Europe.

At harvest time, wheat flows rapidly from the country to the main grain ports because of limited on-farm and internal storage and because this provides immediate cash returns to the producer. Wheat is the first crop harvested and available for export.

The National Grain Board plays the major role in wheat export sale negotiations, but deliveries are generally arranged through the Argentine's private grain companies and co-operatives. In export sales contracts, the shippers use the official wheat grades but may also provide supplementary guarantees of specific factors such as a higher test weight or a minimum

protein level. All export elevators and about half of domestic storage space are owned by the National Grain Board.

The responsibility for grading and issuing certificates of quality for all export grain falls on the National Grain Board. Specifications are officially established for three grades of bread and durum wheats. These grades are regularly established and a fourth grade may be established by the Board with specifications according to season (Fig. 1). The Board makes adjustments in grading tolerances from time to time. In the Argentine system the main grading factors are condition of grain, test weight, % broken or damaged kernels, and % foreign material.

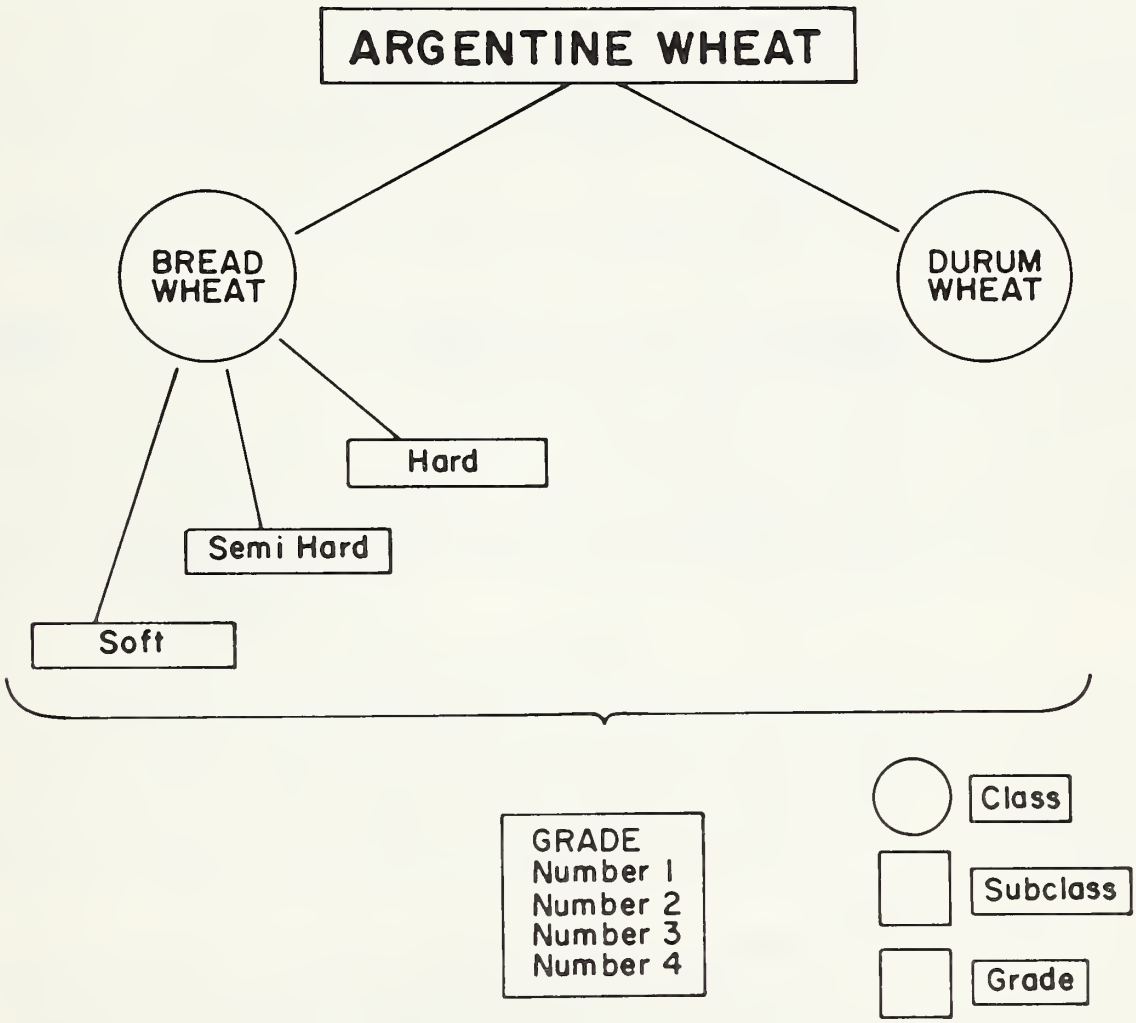

Fig. 1. Classes, subclasses and grades of Argentine wheat

So far as I am aware, the Argentine grading system has not been developed in detail. It is operated under a National Government Act which provides for the mandatory inspection of all wheat traded commercially or shipped for export, and a single agency, the National Grain Board, that is responsible for the inspection of all grain exported. It also provides for a board to establish separate schedules of grades and specifications for

bread and durum wheats.

The National Government Act provides for port terminals to maintain grade identity of receipts and shipments. Mixing is not permitted and the only processing allowed is treatment for infestation control.

The bread wheats are subdivided into three subclasses; hard, semi-hard, and soft. A set of up to four numerical grades in combination with those classifications are applied to each of four production zones. These zones which are defined in the standards for Rosafe, Buenos Aires, Bahia Blanca, and Entre Rios. Typical grade as certified would be, for example, Bahia Blanca, Hard No. 1. Wheats from Rosafe and Buenos Aires zones are sometimes referred to in international trade as Plate wheats.

Annually, the National Grain Board collects grain from each zone, to represent not less than 20% of the estimated production, which is used to establish the official standard sample for each grade. The scheduled grade specifications apply to producer sales, but all commercial transactions subsequent to that require the wheat to be equal to the official standard sample of the grade sold.

In delivery and sale transactions, at the producer level, the Board acts only in cases of arbitration to determine a grade on request from a producer.

Export certification of grades is provided by the National Grain Board which has full responsibility for guaranteeing quality equal to the current standard of the grade. Grade factor information includes the actual protein content of the cargo for grades of bread wheat.

Varietal control is maintained by the Board through producer declaration at country point and by annually listing the acceptable varieties for each zone. Grain of other varieties is simply rejected and traded or sold by sample grade at severe discount. This system of varietal control has served to greatly improve the general quality of Argentina's Plate wheats.

AUSTRALIA

The Australian grading system is operated under state law through completely autonomous state grain handling authorities, each having complete control over purchase, classification or grading, storage, transportation for domestic or export trade and delivery to ocean vessels. Each state has representation on the Australian Wheat Board, which has no statutory authority or power relative to the grading of wheat but advises the states in setting up their grade specifications in accordance with the requirements of the international markets.

Although the Australian grading system is formalized, it does not have statutory status. It is flexible and the grades may be changed each year in accordance with the quality of the crop or of market demands.

In October 1974, the Australian Wheat Board announced the introduction of a change in the names of the grades of Australian wheat. The new names took effect in time to be applied to the grading of producer deliveries in the 1974-75 crop year. The designation F.A.Q., Fair Average Quality, which for many years had described the major class of wheat exported from Australia was discontinued and replaced by A.S.W., Australian Standard White. At the same time, the name "Offgrade" was replaced by two new classifications, Australian General Purpose and Australian Feed. Australian wheat is now classified into six major classes; Prime Hard, Hard, Standard White, Soft,

General Purpose, and Feed (Fig. 2). For any crop year, each class may be subdivided into a number of grades on the basis of test weight, variety (i.e. area or state of production) and in some cases, protein content. The 1974 class name change, however, has not eliminated the previous weakness of the Australian grading system in that there can be significant differences in quality of A.S.W. wheat from different states.

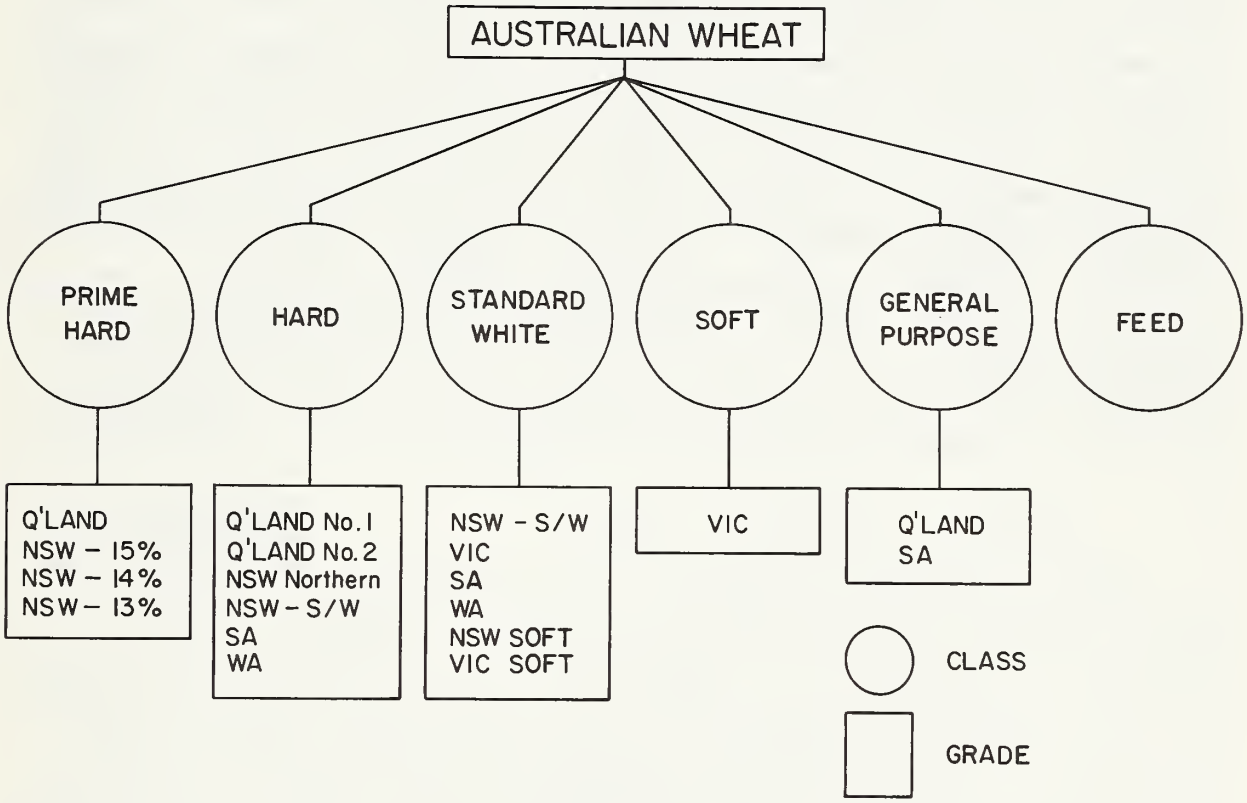

Fig. 2. Classes and grades of Australian wheat

In Australia, inspection is mandatory in all states for all wheat delivered to state handling authorities for export or for domestic use. There is official inspection by a state inspector or classifier at time of producer delivery at the country receiving point. Due to the natural geography of the country, each state's production moves to state-operated ports and this facilitates a rather simple segregation of grades through the state-controlled handling system.

A dual standard system operates effectively with producer deliveries graded according to defined minimums and tolerances, and export shipments graded according to the established seasonal standard sample for each grade.

These standards are composited by the state handling authorities from early harvest new crop grain. Segregation by grade is maintained from country receiving points right through to delivery export vessels.

At the producer delivery level, there is no provision for arbitration of a state inspector's decision, although supervisory staff will investigate complaints. All wheat to be received must conform to the grade specifications as to cleanliness, freedom from weed seeds (30 per pint), and moisture content (not more than 12%). If the grain does not meet specifications it is refused because neither inland elevators nor port terminals are equipped to clean or dry large quantities of grain. Wheat is not necessarily marketed at the grade it is received. Reclassification, both upgrading and downgrading, can occur based on comprehensive post-receival testing of running bulk samples from individual country silos.

At port elevators wheat is received, stored, and shipped according to original classification and normally the only processing is fumigation for infestation control. Export inspection is used to check the state classification. Certificates issued by the State Departments of Agriculture show grade information but not protein content. The Australian Wheat Board bears full responsibility for guaranteeing shipments equal to the season's standard sample for each grade.

Some varietal control is exercised in states where Prime Hard and Hard grades are produced. This is accomplished by the state grain handling authority specifying, annually, those named varieties acceptable for those grades and accepting in any other grade only registered and approved varieties. Growers are required to declare the variety when seeking Prime Hard or Hard classifications at the receiving depot.

CANADA

Canada's grading system operates under a national government act, the Canada Grain Act, which provides for the mandatory official inspection of grain produced in Western Canada during movement to domestic or export markets and permissive or "on-request" inspection of Canada's eastern grain production used domestically. Operators of terminal and transfer elevators, who are licensed under provisions of the Act, must permit official inspection of all Canadian grain when it is being discharged for direct export. A single agency, the Canadian Grain Commission, is responsible for control in the physical handling of grain and for uniform application of inspection and grading standards.

Separate grade schedules are established under the Act for each class of wheat, such as Red Spring, Amber Durum, Soft White Spring and Red and White Winter Wheats (Fig. 3). The separate schedules are designed to provide individual grade tolerances of various factors, such as purity of class and foreign material and are closely related to the ranges of quality desired by the end-users. No. 1 and No. 2 Canada Western (C.W.) grades of Red Spring Wheat are segregated on the basis of protein content and this provides for guaranteed levels of protein, for example 13.5, 12.5, and 11.5% at 13.5% moisture.

In the Canadian system, the main grading factors are bushel weight, variety, soundness, and foreign material. Protein content is not a grading factor. Under the system, grain is evaluated on the basis of visual assessment of factors related to quality and grades are assigned in accordance with

specifications established under the Canada Grain Act.

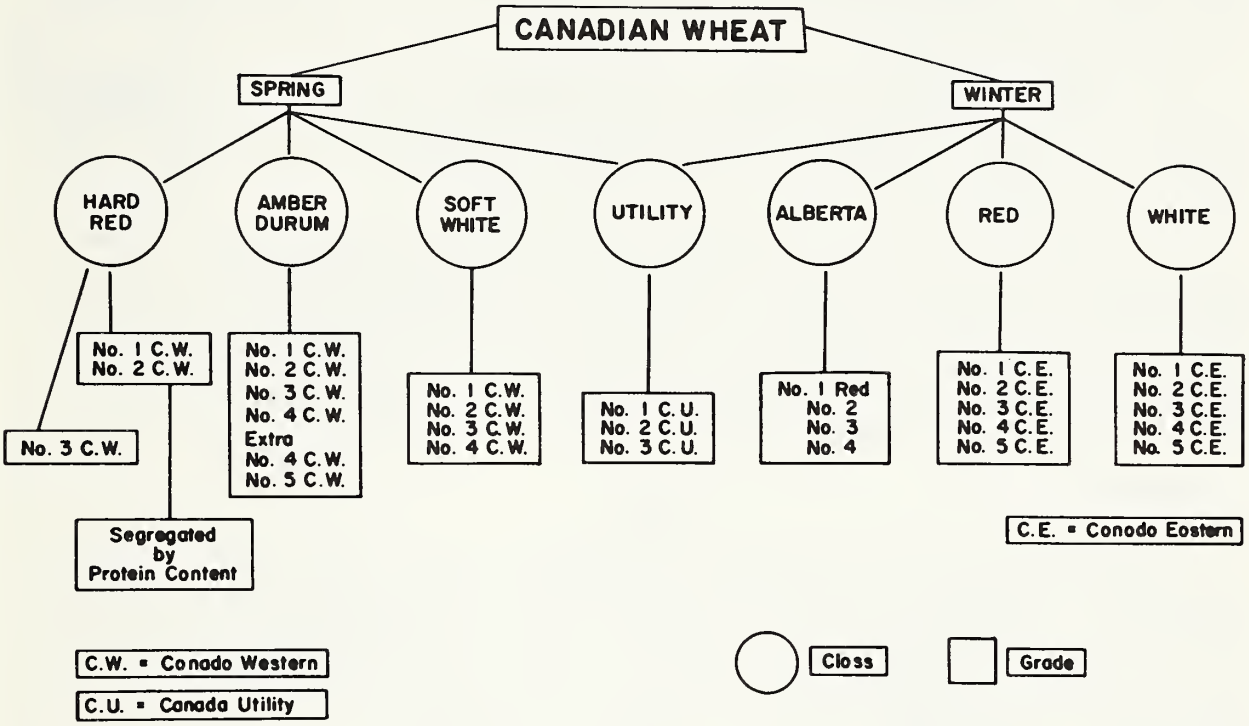

Fig. 3. Classes and grades of Canadian wheat

New crop harvest samples are collected early in each new crop year and from these a producer-oriented Standards Committee recommends primary standard samples for each statutory grade of grain and export standard samples for the higher grades of Red Spring Wheat and Amber Durum Wheat. The primary standards are used as judgement guides in all inspections up to and on receipt of wheat at terminal and process elevators. The export standard sample governs the grading of all outward wheat shipments from terminal elevators. This dual standard system ensures that the quality of the grain shipped out of terminal elevators will be reasonably close to the quality average of terminal receipts of any grade.

The Canadian statute provides the option, at the primary or country elevator, for the farmer to obtain settlement on a negotiated grade and dockage basis, or, on the request of the producer, having official grading of an agreed sample by an inspector of the Canadian Grain Commission.

The terminal elevator may only receive wheat that has been officially

inspected, and all receipts are graded according to the primary standard and dockages are assessed for all readily removable material. All wheat shipped out of the terminal must be commercially clean and essentially free of dockage. Foreign matter tolerances are specified in the specifications for the export standard samples as recommended by the Committee on Grain Standards. Final designation of all standards rests with the Canadian Grain Commission. Mixing of wheat in grades No. 1 C.W. and No. 2 C.W. Red Spring is not permitted, ensuring that quality of grade is maintained through to the purchasers and importers.

All wheat exported is shipped on the basis of a Certificate Final at port of loading, with the Canadian Government guaranteeing that the shipment is equal to, or better than, the export standard sample for the grade. The certificate states the grade and guaranteed protein level but no other quality information.

An important part of the Canadian grading system is the varietal standard named for the top grades of Red Spring and Amber Durum Wheats. This facilitates the control of the varieties that are grown and has a built-in desirable effect on the quality of the wheat. Varieties of the same wheat class can be excluded from top grades if they have not been declared equal in quality to the statutory varietal standard (Marquis for Hard Red Spring Wheat and Hercules for Amber Durum Wheat).

FRANCE

The development of the European Common Market has considerably changed the marketing of wheat in Europe. Consequently, the quality of wheat for marketing -- still in constant evolution -- has been considerably improved.

Only public state organizations are involved in the legislative control and protection of wheat quality in France. The Office National Interprofessional des Cereales (O.N.I.C.) under the Ministry of Agriculture is responsible for quality control relative to marketing.

The O.N.I.C. buys and sells about one-third of the annual cereal production in France. This operation is called "intervention" and is designed to regulate the market. The technical conditions and price of "intervention" are determined each year, in consultation with other members of E.E.C.

The O.N.I.C. has instituted a system of wheat grading that is ratified by an official certificate. This grading, or quality definition, for bread wheat comprises four subclasses determined by protein content and sedimentation test, and, for each subclass, two grades (Fig. 4). The durum wheat has four numerical grades.

Generally, a minimum standard of quality is established for wheat. Wheat with quality higher or lower than this standard is valued basis the "intervention" price. Quantities of wheat meeting the standard grade requirements, but not eligible for the two established grades, are classified but designated "out of grade".

Wheat varieties that can be cultivated in France are controlled. Satisfactory new and existing varieties are officially registered and reproduced by specialized producers before being sold to farmers as seed. This procedure is designed to encourage the use of pure varieties of high germination capacity, but as the E.E.C. varietal standards are somewhat lower than those of France, a number of new varieties have been recently introduced with high agricultural yield but very poor baking quality.

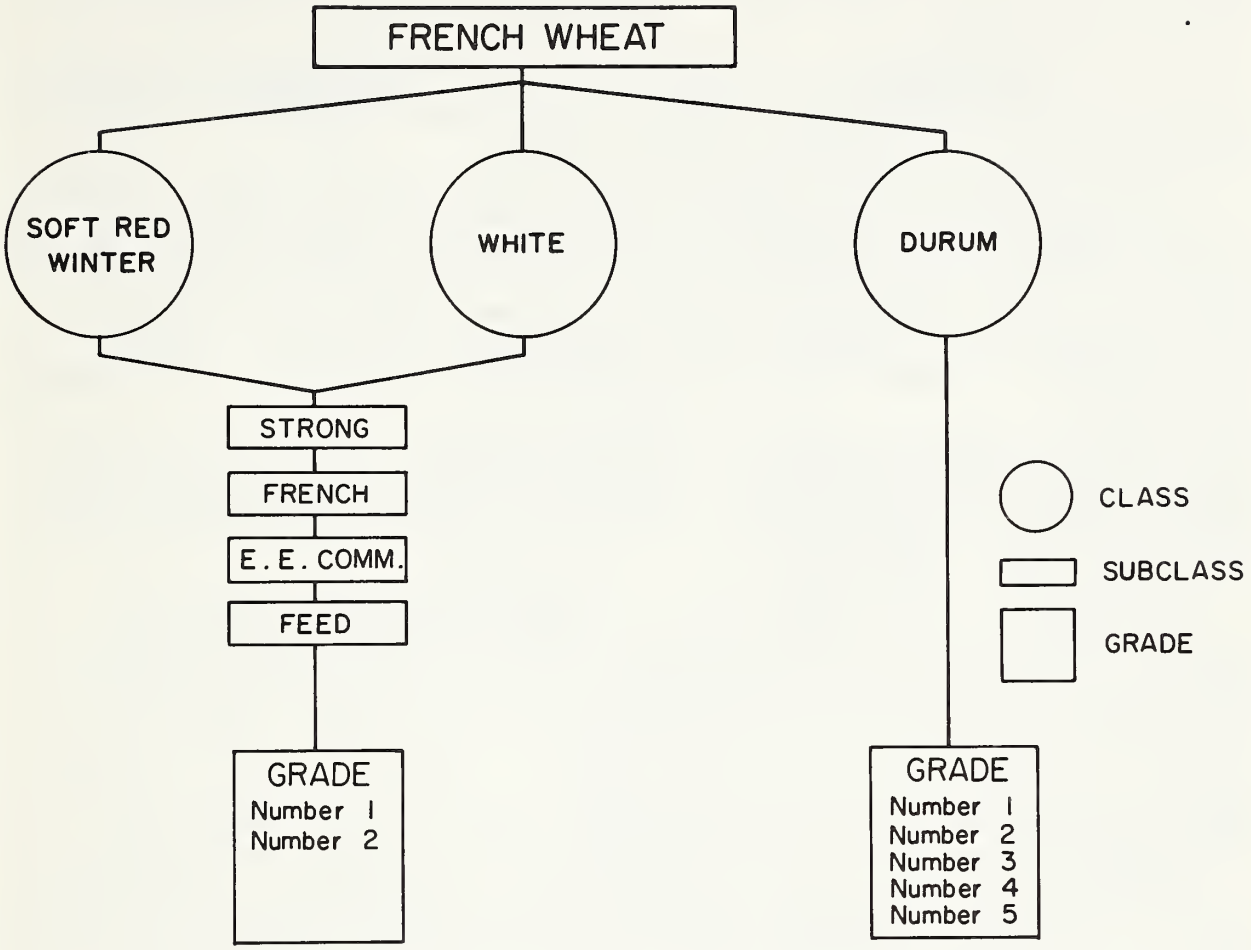

Fig. 4. Classes, subclasses, and grades of French wheat

It should be noted that durum wheat production in France has dropped to a point where the country is barely self-sufficient. Revisions on varietal standards are presently being made in an attempt to increase production to a point where France will again become an exporter of durum wheat.

UNITED STATES

A new Federal Grain Standards Act was pronounced in the U.S.A. effective November 20, 1976. Under the terms of the Act, all grain exported must be officially inspected under Federal authority at port of loading. Only Federal Grain Inspection Service (F.G.I.S.) or delegated State officials will be permitted to perform original export inspections. Similarly, only F.G.I.S. or delegated state officials can certify the weights of export grain shipments.

The Federal Grain Inspection Service was established in the U.S. Department of Agriculture under the new Act. Its primary task is the implementation of the U.S. Grain Standards Act, to assure that the grain

standards are relevant to market value and end use and are uniformly applied throughout the U.S.A., and to provide unbiased, accurate, and efficient inspection services.

In the inland marketing of grain for domestic consumption, however, the F.G.I.S. role will be mainly of a supervisory nature over agencies designated to perform inspection services. Appeals on such inspections will be performed by the Federal Service on request only.

The formulation of new national controls within the U.S. grain industry will have broad impacts on U.S. grain handling and marketing systems. Some of the changes which are anticipated include the use of protein content as a grading factor in wheat, improved control over varieties for production, revisions to grain standards in relation to market requirements, and greater accountability by the industry in grain handling and marketing. Some of these changes were made effective May, 1977; other changes will follow shortly afterwards.

Although there is no mandatory inspection of wheat received into terminal or seaboard elevators, inspection is generally applied to all receipts from nonline shippers (other companies). There are no mixing prohibitions of any kind in terminals. Wheat may be shipped with dockage, assessed to the nearest 0.5%, (0.4 = 0 and 0.9% = 0.5%).

U.S. wheats are divided into six classes (Fig. 5). Some classes are further subdivided into as many as four subclasses. A single schedule of numerical grades and grade specifications applies to all classes of wheat. The grading system is a single standard system and one set of specifications for any given grade applies equally at the primary and export inspection levels.

Test weight, heat and other kernel damage, shrunken and broken kernels, admixtures of contrasting classes of wheats, wheats of other classes, and foreign material are the grading factors in the U.S. wheat grading system.

Export inspection certificates are issued by the Federal or officially delegated export grain inspection service, thereby establishing responsibility for quality of export shipments. Virtually any factor information may be shown on the inspection certificate on request of the exporter, including protein content.

Varietal restrictions are not imposed through the medium of inspection and grading of grain. The licensing of varieties in the U.S.A. is under state control. There is little or no restraint on the production or mixing-off of varieties of different quality.

SUMMARY

Tables 1 to 6 give the specifications and tolerances of the major factors that are used to establish the numerical grade. A careful examination of these tables indicates some significant differences in the specifications for the top grades of bread wheat among the five countries considered.

ACKNOWLEDGMENT

This article was prepared in consultation with Mr. V. Duke, Director, Inspection Division, Canadian Grain Commission, Winnipeg, Canada.

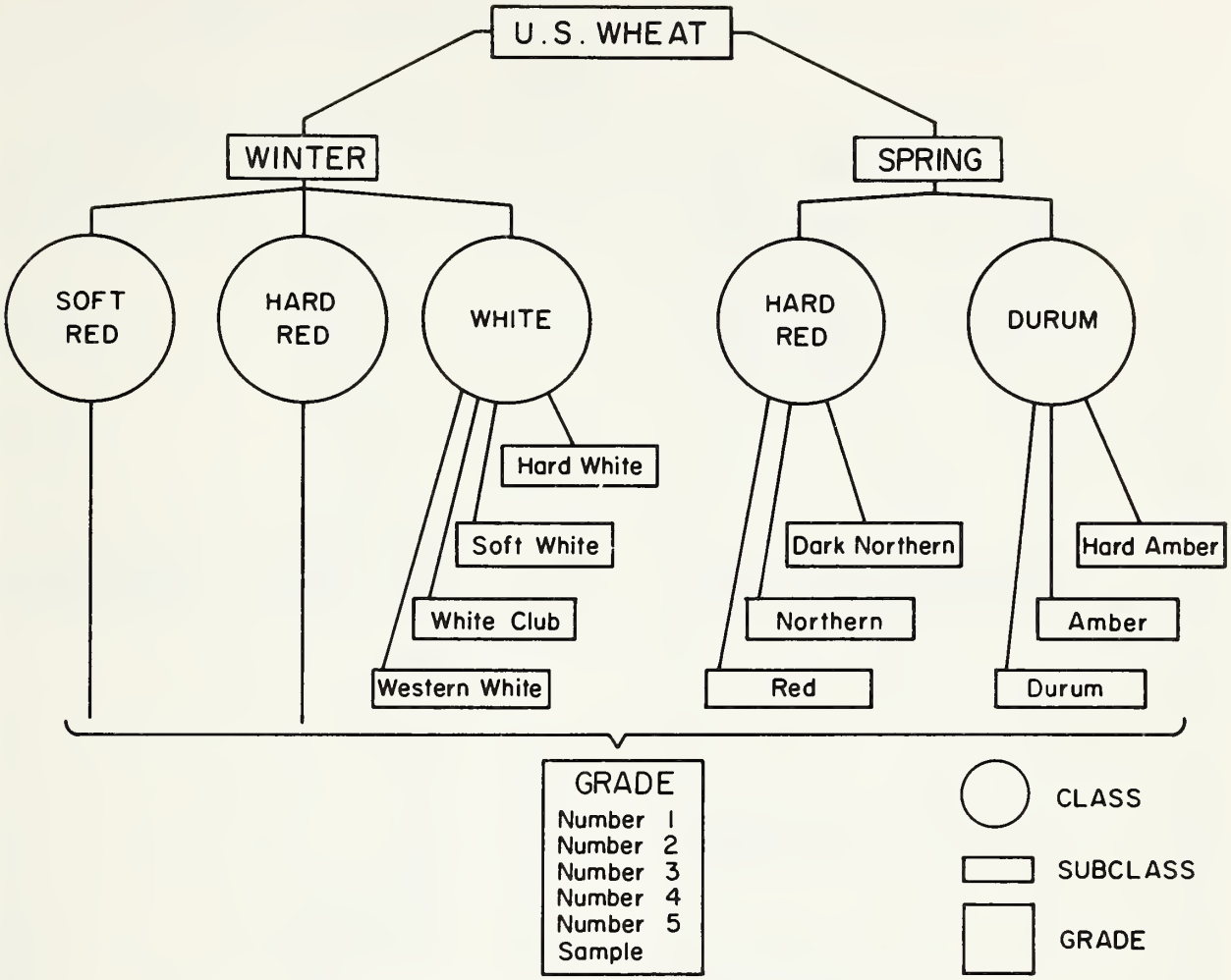

Fig. 5. Classes, subclasses, and grades of U.S. wheat

Table 1. Highest Quality Bread Wheat Grades: Minimum Test Weight Specifications* (Kilograms/Hectoliter)

Country	Grade No. 1	No. 2	No. 3	Class
Argentina	78.0	75.0	72.0	Hard Red
Australia	74.0 No. 1 Prime Hard	74.0 No. 1 Hard	71.1 No. 2 Prime Hard No. 2 Hard	Hard White
Canada	79.1	78.5	75.8	Hard Red Spring
France	76.0	75.0	--	Strong White
United States**	74.6 77.2	73.4 74.6	70.8 72.1	Hard Red Spring Hard Red Winter

* Basis Cleaned Sample

** "Heavy" can be added to grade description if
77.2 kg/hl or more for Hard Red Spring and
79.8 kg/hl or more for Hard Red Winter wheat.

Table 2. Maximum Moisture in Straight Grades

Country	Bread
	%
Argentina	-
Australia	12.0
Canada	14.5
France	15.5
United States	-

Table 3. Protein Content (N X 5.7), Guaranteed Levels

Country	% Guaranteed	Moisture Basis	Remarks
Argentina	-	Dry Matter	
Australia	15.0 14.0 13.0	Natural	Nos. 1 and 2 Prime Hard Only
Canada	13.5 12.5 11.5	13.5%	Nos. 1 and 2 Canada Western Red Spring
France	11.25 10.4 9.5 8.65	13.5%	Soft Red Winter and White Spring
United States	Contracted	14.0%	Guarantees Contracted

Table 4. Varietal Standards

Country	
Argentina	Acceptable varieties for higher grades established annually
Australia	Variety requirements established for each State
Canada	Marquis
France	Acceptable varieties established in consultation with E.E.C.
United States	None

Table 5. Wheats of Other Classes*

Grade	No. 1		No. 2		No. 3	
Country	Durum	Total	Durum	Total	Durum	Total
Argentina	1.0	-	2.0	-	3.0	-
Australia	-	60	-	60	-	60
Canada**	0.2	1.5	0.5	0.4	2.5	7.5
France	-	-	-	-	-	-
United States	1.0	3.0	2.0	5.0	3.0	10.0

* Percent except Australia where the figure is in seeds per litre.

** Total includes varieties not equal to standard variety.

Table 6. Limits of Foreign Material*

Grade	No. 1		No. 2		No. 3	
Country	Matter Other Than Cereal Grains	Total	Matter Other Than Cereal Grains	Total	Matter Other Than Cereal Grains	Total
Argentina	4.0	4.0	6.0	6.0	9.0	9.0
Australia	3	63	3	63	3	63
Canada	0.15	0.4	0.15	0.6	0.2	1.0
France	0.5	2.0	1.0	3.0	-	-
United States	0.5	0.5	1.0	1.0	2.0	2.0

* Percent except Australia where the figures are in seeds per litre.

INFLUENCE OF PLANTING DATE AND CROP YEAR ON THE MILLING AND BAKING CHARACTERISTICS OF CONVENTIONAL HEIGHT AND SEMIDWARF HARD RED SPRING WHEATS

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ABSTRACT

Two conventional height hard red spring (HRS) wheats (Chris and Waldron) and two semidwarf HRS wheats (Olaf and WS 1809) were planted during two crop years (1974 and 1975) on four planting dates one week apart. The kernel and milling data for the 1974 crop year samples (Table 1) and the 1975 crop year samples (Table 2) for the four cultivars showed the average test weight, 1000 kernel weight, and flour extraction were lower for the 1974 crop samples than the 1975 crop samples, but had a higher percentage of medium kernels and protein content. The flour and baking data for the 1974 crop year samples (Table 3) and the 1975 crop year samples (Table 4) showed the average flour ash content higher, mixing time longer, and the bake absorption and loaf volume lower for the 1974 crop year samples than the 1975 crop year samples, while the mixogram patterns were the same. Ranges among the planting dates for the milling, flour, and baking characterization values (Table 5) of the two crop years showed that Chris had the greatest range for 3 values; WS 1809 for 6 values; and both Olaf and Waldron for 7 values. This would indicate that the recently developed varieties had a greater genetic-environmental interaction which would minimize the predictability of the quality factors. This is verified by the averages of the coefficients of variations (Table 6) which were 3.26 for Chris, 4.23 for WS 1809, 4.74 for Waldron, and 5.14 for Olaf.

Also, two durum wheat cultivars (Table 7) grown at El Centro, California, were planted on four consecutive dates approximately one week apart. Correlation matrix between test weight (TW), 1000 kernel weight (KW), medium size kernels (MD), semolina extraction (SEEX), spaghetti color score (VI), spaghetti firmness (FR), and wheat protein content (PR) showed highly significant correlations (1% level) between TW vs KW (0.87), TW vs VI (-0.73), KW vs VI (-0.82), MD vs VI (0.77), and SEEX vs VI (-0.90); and significant correlations (5% level) between KW vs SEEX (0.63), KW vs MD (0.61), MD vs SEEX (-0.71), and VI vs FR (-0.68). Although the data showed that certain quality factors were correlated, the data indicated that the Modoc cultivar was influenced more by the planting date than Mexicali, especially the final evaluation score.

Thus, planting date can have an appreciable influence on the quality of the wheat sample for both durum and hard red spring wheats, depending on the cultivar.

TABLE 1

KERNEL AND MILLING CHARACTERIZATION FOR 1974 CROP FOR FOUR HARD RED SPRING WHEAT CULTIVARS FOR FOUR DIFFERENT PLANTING DATES

VARIETY	PLANTING DATE	TEST WEIGHT lb/bu	1000 KERNEL WEIGHT g	MEDIUM KERNELS %	WHEAT PROTEIN[a] %	FLOUR EXTRACTION %
Chris	1	56.9	20.3	81	16.0	66.8
Chris	2	56.8	20.7	82	15.7	67.1
Chris	3	54.3	18.6	77	17.1	65.7
Chris	4	56.2	19.5	85	17.4	67.2
Olaf	1	58.1	25.0	87	15.6	66.5
Olaf	2	57.4	26.1	88	15.7	66.9
Olaf	3	55.9	23.5	88	17.1	66.1
Olaf	4	57.9	24.3	92	17.3	66.6
Waldron	1	56.9	24.7	88	16.7	66.4
Waldron	2	56.9	24.6	89	15.7	66.8
Waldron	3	53.7	21.7	86	17.7	65.9
Waldron	4	56.0	24.0	90	17.4	67.5
WS 1809	1	58.9	23.5	97	15.1	65.6
WS 1809	2	59.0	22.4	86	14.6	66.1
WS 1809	3	56.5	21.1	82	15.9	64.6
WS 1809	4	57.4	20.2	84	15.7	65.6

TABLE 2

KERNEL AND MILLING CHARACTERIZATION FOR 1975 CROP FOR FOUR HARD RED SPRING WHEAT CULTIVARS FOR FOUR DIFFERENT PLANTING DATES

VARIETY	PLANTING DATE	TEST WEIGHT lb/bu	1000 KERNEL WEIGHT g	MEDIUM KERNELS %	WHEAT PROTEIN[a] %	FLOUR EXTRACTION %
Chris	1	58.8	21.7	88	15.3	67.4
Chris	2	60.2	24.0	92	15.3	67.0
Chris	3	60.4	27.0	91	15.7	67.3
Chris	4	61.1	27.5	87	16.0	66.4
Olaf	1	60.2	28.4	88	14.2	67.0
Olaf	2	61.3	32.1	84	14.6	66.4
Olaf	3	61.0	31.4	68	15.7	66.9
Olaf	4	61.6	31.7	62	15.5	65.7
Waldron	1	59.0	26.4	84	14.5	67.8
Waldron	2	59.9	27.2	81	14.8	67.4
Waldron	3	60.3	29.2	63	15.3	67.9
Waldron	4	60.9	30.8	52	15.0	67.2
WS 1809	1	60.0	25.1	89	14.5	68.2
WS 1809	2	60.8	26.2	91	14.2	67.8
WS 1809	3	59.3	26.2	91	15.1	68.2
WS 1809	4	59.5	23.5	89	14.8	68.5

a/ 14% moisture basis.

TABLE 3

FLOUR AND BAKING CHARACTERIZATION FOR 1974 CROP FOR
FOUR HARD RED SPRING WHEAT CULTIVARS FOR FOR DIFFERENT PLANTING DATES

Variety	Planting Date	Flour Ash[a/b/]	Mixogram Pattern[c/]	Bake Absorption	Mixing Time	Loaf Volume
		%		%	min	cc
Chris	1	.41	4	63.3	3.75	895
Chris	2	.42	4	62.9	3.75	870
Chris	3	.42	4	64.1	3.75	870
Chris	4	.39	4	63.9	3.75	890
Olaf	1	.39	7	60.0	6.50	875
Olaf	2	.40	7	59.7	6.00	935
Olaf	3	.41	7	63.7	6.25	920
Olaf	4	.38	8	65.3	7.50	965
Waldron	1	.43	6	62.9	5.75	1000
Waldron	2	.43	6	63.7	5.75	985
Waldron	3	.46	7	68.3	6.25	1035
Waldron	4	.41	7	67.6	6.50	1090
WS 1809	1	.40	5	62.9	5.50	980
WS 1809	2	.39	6	62.0	5.00	965
WS 1809	3	.42	5	61.7	5.25	925
WS 1809	4	.43	5	63.4	4.75	900

TABLE 4

FLOUR AND BAKING CHARACTERIZATION FOR 1975 CROP FOR
FOUR HARD RED SPRING WHEAT CULTIVARS FOR FOUR DIFFERENT PLANTING DATES

Variety	Planting Date	Flour Ash[a/b/]	Mixogram Pattern[c/]	Bake Absorption	Mixing Time	Loaf Volume
		%		%	min	cc
Chris	1	0.39	4	62.5	3.65	1000
Chris	2	0.38	5	65.0	4.13	973
Chris	3	0.41	5	67.0	4.50	940
Chris	4	0.44	5	67.0	4.38	965
Olaf	1	0.42	8	62.5	6.66	955
Olaf	2	0.35	7	62.3	6.75	1013
Olaf	3	0.36	8	66.2	6.75	990
Olaf	4	0.36	6	65.7	6.13	1000
Waldron	1	0.4[illegible]	6	62.5	5.25	945
Waldron	2	0.37	5	62.6	4.88	983
Waldron	3	0.36	5	62.9	4.66	960
Waldron	4	0.36	5	63.7	4.13	965
WS 1809	1	0.37	6	60.1	5.25	933
WS 1809	2	0.35	5	63.1	4.75	910
WS 1809	3	0.35	6	64.9	5.50	920
WS 1809	4	0.33	7	64.6	6.88	940

a/ 14% moisture basis. b/ Ash at 65% extraction.

c/ 1 weak to 10 very, very strong.

TABLE 5

RANGES IN MILLING, FLOUR, AND BAKING CHARACTERIZATION VALUES

VARIETY	TW	KW	MK	PRO	EXT	ASH	MP	ABS	MT	LV
1974 Crop										
Chris	2.6	2.1	8	1.7	1.5	.03	0	1.2	0.00	25
Olaf	2.2	2.6	5	1.7	0.8	.03	1	5.6	1.50	90
Waldron	3.2	3.0	4	2.0	1.6	.05	1	5.4	0.75	105
WS 1809	2.5	3.3	15	1.3	1.5	.04	1	1.7	0.75	80
1975 Crop										
Chris	2.3	5.8	5	0.7	1.0	.06	1	4.5	0.85	60
Olaf	1.4	3.7	26	1.5	1.3	.07	2	3.9	0.62	58
Waldron	1.9	4.4	32	0.8	0.7	.06	1	1.2	1.12	38
WS 1809	1.5	2.7	2	0.9	0.7	.04	2	4.8	2.13	30

TABLE 6

COEFFICIENT OF VARIATION OF KERNEL, MILLING, FLOUR, AND BAKING CHARACTERIZATION VALUES OF FOUR HARD RED SPRING WHEAT CULTIVARS FOR FOUR PLANTING DATES AND TWO CROP YEARS

Character	Year	Chris	Olaf	Waldron	WS 1809
Test Weight	1974	1.86	1.50	2.19	1.81
	1975	1.39	0.85	1.15	0.97
Kernel Weight	1974	4.07	15.38	5.11	5.76
	1975	9.39	4.74	6.06	4.09
Medium Kernels	1974	3.52	2.16	1.68	6.65
	1975	2.09	14.31	18.76	1.11
Protein	1974	4.33	4.74	4.56	3.34
	1975	1.89	3.99	1.96	2.29
Flour Extraction	1974	0.77	0.43	0.55	0.85
	1975	0.58	0.77	0.42	0.36
Ash at 65% Ext.	1974	2.99	2.83	4.13	3.86
	1975	6.18	7.44	6.59	4.04
Mixogram Pattern	1974	0.00	5.97	7.69	8.25
	1975	9.12	11.44	8.25	11.79
Bake Absorption	1974	0.79	3.85	3.59	1.09
	1975	2.83	2.78	0.75	3.01
Mixing Time	1974	0.00	8.68	5.36	5.45
	1975	8.46	4.57	8.79	14.47
Loaf Volume	1974	1.29	3.52	3.93	2.43
	1975	3.61	2.91	3.37	2.98

TABLE 7

DURUM QUALITY EVALUATION

1976 CROP PLANTING DATE STUDY

VARIETY	TW[1] #/Bu	KW g	LG %	MD %	SM %	PR %	SEEX %	SP	DU	VI	FR	RE %	VAL[2]
AVERAGE-BLEND	63.0	46.8	55	42	3	13.2	56.9	32	126	8.8	8.35	4.7	4
DATE OF PLANTING													
MEXICALI-1	61.7	51.5	64	32	4	12.9	59.6	30	125	8.5	8.55	2.1	4
MEXICALI-2	62.4	51.3	72	25	3	12.9	59.0	30	125	8.5	7.93	3.4	4
MEXICALI-3	62.4	50.5	61	36	3	13.0	58.4	33	120	8.5	8.60	5.6	4
MEXICALI-4	60.8	43.7	56	40	4	12.8	57.8	23	130	9.0	7.95	3.9	4
MODOC-1	64.5	46.7	58	40	2	13.4	57.8	40	125	8.5	8.12	4.5	3
MODOC-2	64.2	44.6	58	40	2	13.5	54.8	33	130	9.0	8.66	6.0	4
MODOC-3	64.4	44.2	37	60	3	13.5	54.8	37	125	9.0	9.14	6.0	3
MODOC-4	63.8	42.0	30	67	3	13.5	53.2	27	130	9.5	7.84	6.1	1

1/ TW = Test Weight; KW = 1000-Kernel weight; LG = Large kernels; MD = Medium kernels; SM = Small kernels; PR = Wheat protein (14% m.b.); SEEX = Semolina extraction; SP = Number of specks in semolina per 64.5 sq cm; DU = Semolina color; VI = Spaghetti color; FR = Cooked spaghetti firmness in g cm; RE = Cooked spaghetti residue; MG = Milling deficiency based on percent semolina extraction.

2/ VAL = Final evaluation; 1 = No promise; 2 = Little promise; 3 = Some promise; 4 = Good promise.

IMPROVEMENT OF WHEAT PROTEIN QUALITY BY GERMINATION

M.T. Nielsen (1), R.E. Meade (2), G.M. Paulsen (1) and R.C. Hoseney (3)

SUMMARY

Information published before 1976 relative to changes in protein of cereal grains other than wheat during germination led to a project to investigate the effect of germination on wheat protein quantity and character. While our work was in process (1976) Dalby & Tsai (1) published results with germinating wheat. Our results confirm the increases in certain amino acids, especially lysine. Three wheat varieties were germinated for various (0-10 day) periods at three different temperatures (10, 20 and 30 C). Samples were preserved and analyzed to determine:

Dry matter loss
Water-soluble protein
Heat-stable protein
Change in amino acid composition
Change in nitrogen content

Results from these analyses are reported in appended Tables and indicated that lysine content may be increased by germination, to a level that would no longer be limiting. Dry matter loss was not significant except at 30°C and between 4 and 7 days germination time. Both "water-soluble" and "heat-stable" protein increased.

Generally, results showed favorable potential for adaptation to a "wet-milling" process for wheat and a protocol was developed and proposed with a request for funds to support further investigation. The proposal was designed to acquire information pertinent to application of a germination phase prior to "wet-milling" wheat. Funds requested were not approved and "rationale" for rejection is stated.

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REVIEW OF LITERATURE

Most cereal proteins are deficient in one or more essential amino acids. Wheat grain protein is particularly deficient in lysine. Much effort has been expended on increasing the lysine content of wheat grain by breeding, but this effort has been only partially successful. Studies with other cereals, however, suggest that protein quality of wheat can be improved by brief germination of the grain.

The only report on effect of germination on wheat protein quality was by Dalby and Tsai (1). Lysine as a percent of sample weight increased approximately 40% during a germination time of five days at 28 C. Prolamine decreased markedly and protein increased slightly during the same time.

Two studies with barley bear directly on the problem of increasing limiting essential amino acid contents of grains by germination. Folkes and Yemm (2) showed that during a 10-day germination period at 22.5 C, lysine content in germinating barley increased an average of 65% over that in ungerminated grain. The percentage increase in lysine was directly proportional to the grain protein content, being 34% for 9.1% protein grain, 68% for 11.4% protein grain, and 86% for 14.2% protein grain. Most of the increase in lysine occurred in the embryo; little occurred in the endosperm. Similar results were reported by Smith (4). Germination increased the proportions of lysine, alanine, aspartic acid, and glycine, but decreased the proportions of cystine, glutamic acid and proline in barley grain. Lysine increased from 4.42% to 6.05% in low-protein grain after six days' germination (37% relative increase), and from 3.86% to 5.86% in high-protein grain after seven days' germination (52% relative increase). Smith (4) suggested that the low level of cystine in germinated barley might limit the nutritional value of the grain.

Studies with corn by Tsai, Dalby, and Jones (5) showed similar trends as in barley by the above workers. In normal-lysine corn grain during a 5-day germination period, lysine and tryptophan increased in the embryo and whole kernel, but decreased in the endosperm. The increase was about 66% for lysine in the whole kernel.

Although protein content of grains has long been considered to decrease steadily during germination(6), that is not always the case. In the above-mentioned study with corn(5), protein in the whole kernel remained nearly constant over five days. However, there was a shift between endosperm and embryo protein contents. In wheat, soaking grain in water at 10 C for two

days increased the water-soluble endosperm proteins (3). This was thought to be at the expense of ethanol-soluble proteins (gliadins).

The literature reports many factors that influence amino acid and protein metabolism in germinating grain. Among these are time, temperature, air (oxygen), light, reduced (ammonium) nitrogen salts, growth regulators, humigity, and grain protein content.

MATERIALS AND METHODS

Five-hundred-grain samples of 'Chris' (hard red spring), 'Sage' (standard protein hard red winter), and 'Lancota' (high protein hard red winter) wheats were weighed and surface-sterilized. Sterilization was by soaking the seeds in formalin solution (16 ml 40% formaldehyde and 4 ml 100% methanol per liter) for 15 minutes followed by thorough rinsing with distilled water.

Samples of each wheat variety were germinated at 10, 20, and 30 C for 0, 0.5, 1, 2, 4, 7, and 10 days. For each temperature-time combination, duplicate 500-grain samples of each variety were placed on double sheets of whatman no. 4 filter paper in 2-liter opaque styrene containers. The paper was moistened throughout the germination period with distilled water. Germination was done in darkness for the indicated periods.

One set of each duplicate temperature-time combination samples was quick-frozen at the end of the germination period. The samples were lyophilized (freeze-dried) and weighed to determine dry matter loss during germination. Samples were ground through a 20-mesh sieve and submitted for amino acid analyses.

The second set of each duplicate temperature-time combination samples was frozen and held for total water-soluble protein and heat-stable water-soluble protein analysis. Water soluble protein was extracted by homogenizing the thawed samples in 20 ml of water in a Virtis high-speed homogenizer for two 1-minute periods. Homogenized samples were diluted to 100-ml volume. Thirty ml of each sample was centrifuged at 3000 x g for 5 minutes. Five ml of supernatant was removed for water-soluble protein analysis. The remainder of the centrifuged samples were heated in a boiling water bath until protein precipitation occurred. They were held at room temperature for 12 hours and then centrifuged at 8000 x g for 5 minutes. Five ml of supernatant was removed for heat-stable protein analysis. All protein analyses were by micro-Kjeldahl.

RESULTS

Appearance of the three wheat varieties germinated at 10, 20, and 30 C is shown in Figure 1. Seeds began to imbibe water and swell within one-half day after germination started. Visible sprouting of "Chris" and "Sage" seeds occurred after two days at 20 or 30 C. Sprouting of "Lancota" was slightly delayed as compared with the other varieties. After ten days' germination, seedlings were well developed in all varieties germinated at the two higher temperatures. At the 10 C temperature, germination was slow and approximated

germination at 30 C after two days.

Significant dry matter loss occurred only at 30 C between four and seven days' germination time (Table 1). Dry matter loss was greater in "Chris" and "Sage" than in "Lancota," which germinated slower.

Water soluble protein increased six-fold in "Chris" and "Sage" and over three-fold in "Lancota" during germination (Table 2). The proportion of "heat-stable" protein likewise increased. Approximately three-fourths of the protein in ungerminated grain and all the protein in ten-day germinated grain was heat-stable. Little of the protein possessed albuminous characteristics.

Changes in protein and amino acid composition of "Sage" and "Lancota" grain during germination are shown in Tables 3-6. Percentage protein in the grain increased at the 7-day and 10-day germination times (Tables 3, 4, 5). The total amount of protein in the grain, however, remained constant in "Sage" germinated at 20 C, but decreased in "Sage" and "Lancota" germinated at 30 C (Table 6). The decrease in total protein was most severe in "Sage" germinated at 30 C for ten days and may be an abnormality caused by lack of replication.

Lysine as a proportion of protein increased maxima of 30% in "Sage" germinated at 20 C, 62% in "Sage" germinated at 30 C, and 50% in "Lancota" germinated at 30 C for ten days (Tables 3, 4, 5). Tryptopahn in the latter increased 21%. The total amount of lysine per sample increased maxima of 30% in 'Sage' germinated at 20 C for ten days, 50% in 'Sage' germinated at 30 C for seven days, and 19% in 'Lancota' germinated at 30 C for ten days (Table 6).

DISCUSSION

Germination increased nutritional quality in terms of lysine content as effectively in wheat grain as in barley and corn grain. Our results with wheat were comparable to those reported by Dalby and Tsai (1) with wheat and by Folkes and Yemm (2) and Smith (4) with low-protein barley. Our results with the high-protein wheat, 'Lancota', did not show greater lysine increase in that variety than in the normal-protein wheat, 'Sage'. That was unlike the difference between low and high-protein barley (2, 4). Two factors might cause that difference. 'Lancota' germinated slower than the other varieties, which would not have permitted time for maximum lysine increase. Also, Folkes and Yemm (2) and Smith (4) compared similar varieties that had different protein contents as a result of differential nitrogen fertilization. We compared different varieties that had different protein contents as a result of genetic potential.

The lysine content of wheat in our studies was elevated sufficiently so that amino acid would likely no longer be limiting. Methionine might become limiting, but germination had no effect on methionine. Cystine analyses were not made so it is unknown if that amino acid might become limiting in wheat as Smith (4) suggested it might in barley.

Further research is needed to optimize amino acid recovery in germinated wheat. The 10-day germination time we used equalled or exceeded the 6, 7, and 10-day times used for barley (2, 4) and the 5-day time used for corn (5). However, lysine was still increasing in wheat even after ten days. Germination rate, dry matter loss, and amino acid recovery can likely be manipulated with plant growth regulators. Gibberellic acid accelerates these changes; maleic hydrazide decelerates the changes. Maleic hydrazide might also permit changes in amino acid composition without extensive shoot and root development during germination. Relative amino acid changes in low- and high-protein wheat induced by nitrogen fertilizer should be determined. The fact that lysine increase is greater in high-protein barley than in low-protein barley suggests that adding exogenous reduced nitrogen would increase lysine production during germination. That possibility should be investigated with wheat.

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POSTSCRIPT (Historical Comment, by this Author (R.E.M.) only).

The project reported above was initially proposed as a possible approach to improving protein quality in foods prepared from processed wheat. The suggestion was in a prospectus for a wet-milling process for wheat (as a proliferation of such a process) originated by this author in 1966-67. After the basic wet-milling process had been developed during several years of laboratory and plant scale studies, an encouraging but superficial preliminary literature review relative to germination was made in 1973-74. As a result, the project reported herein was initiated in 1974 as a peripheral development related to the wheat "wet-milling" processes described in U.S. Patent Numbers 3,851,085 (11/26/74) and 3,868,355 (2/25/75) issued to N.E. Rodgers and others, assigned to the Pillsbury Co. These processes separate wheat into various fractions in an aqueous slurry system requiring addition of substantial proportions of water to the dry intact wheat kernels used as feed material, thus necessitating eventual removal of most of this water by mechanical force or evaporation. Since the water added for wet-milling was greater than that anticipated to permit germination, the latter offered a process variation with a potential to improve the nutritional quality of the protein to be recovered possibly without added energy or expense for separating water from the wheat solids. It was, however, recognized that changes might occur which could impede success, such as: loss of protein; impairment of function; or excessive higher cost of end products from changes in components due to microbial, biochemical or chemical activity. At the same time it was considered possible that unanticipated and unpredictable changes might evolve which could be beneficial either to end products or efficiency or even offer unexpected new products.

Subsequent to the study reported above (which appeared to offer a modicum of encouragement) a protocol was proposed early in 1976 for a second phase of exploration designed to emphasize pertinent "limiting condition parameters" and exogenous factors to provide for maximum recovery of desired amino acids (especially lysine) and proteins. The following is an excerpt from the proposed protocol:

The conditions of time and temperature should be extended and increased (within feasible practical limits) to establish the magnitude of the amino acid changes. Treating the germinating grain with an exogenous supply of nutrients and growth regulators should be studied to determine the effects of the treatments on the amino acid and protein content in the grain. In order to more closely monitor the amino acid changes, the location of the lysine increase should be identified. Differences between low-protein grain and high-protein grain should be investigated. Furthermore, studies should be undertaken to determine the feasibility of germinating "endosperm-free" embryos rather than the intact grain. Finally, those conditions and exogenous factors showing the greatest potential should be optimized to allow for the maximum recovery of essential amino acids and protein.

MATERIALS AND METHODS

A standard protein wheat, "Sage," used in the first phase of this research will be surface sterilized to minimize microbial contamination. The grain will be germinated in the dark in Petri dishes containing filter paper moistened with distilled water. Each grain sample will consist of three 100-grain (ca. 3 g. each) replications germinated in separate Petri dishes. After the specified germination periods are complete, the three 100-grain portions will be composited and the percent germination will be calculated. The bulked samples will be freeze-dried, weighed, and analyzed for amino acid content. At 7, 10, and 13 days after the start of germination, grain kept at two temperatures, 30 and 37 C, will be removed for analysis. Also removed at those same times will be samples germinated at 30 C in the presence of thiourea. At this same temperature, grain germinated in the presence and in the absence of gibberellic acid and maleic hydrazide will be removed for analysis at 2, 4, and 7 days after germination starts. Larger samples (three 200-grain portions) will be germinated at 30 C, removed at the 7th and 10th day, and separated into three components (shoots, roots, and seed remnants) for protein and amino acid analyses. In addition, grain which has had the major portion of the endosperm removed by lateral dissection will be surface sterilized, placed in Petri dishes, and kept at 30 C for 2, 4, 7, and 10 days. After that the viability and amino acid content of the embryos will be determined.

Wheat field plots would be fertilized with 0 to 240 pounds of nitrogen per acre. Thses should yield grain with marked differences in protein concentrations. This grain will be used to determine if lysine recovery increases with grain protein concentration in wheat as it does in barley. This study and the one with exogenous thiourea supplied during germination will determine the relationship between nitrogen supply and lysine recovery.

Research to determine the maximum recovery of amino acids and protein will be contigent on the optimization of the above conditions.

The foregoing proposal was not approved. Rationale for the rejection might be surmised as follows:

1. Samples of the sprouted wheat were analyzed to determine whether the gain in lysine occurred as free lysine only. Although results were not clear cut it was believed that much of the lysine (about half) was present as free lysine. Gain in protein lysine thus was not assured.

2. A brief preliminary economic projection may have indicated probable higher cost (60-70%) for germination compared with supplementation with purchased lysine.

3. No obvious recognizable new product applications or satisfaction of known market needs may have been apparent.

4. No assurance had been established that the "Sprouted Wheat" process variation would be readily adaptable to existing facilities and methods available for wet-milling without excessive additional capital cost or process expense.

These criteria are only a guess but experience assures me of their probability. My own preliminary feasibility study projected favorable odds & recommended further investigation along the lines proposed in the subject protocol.

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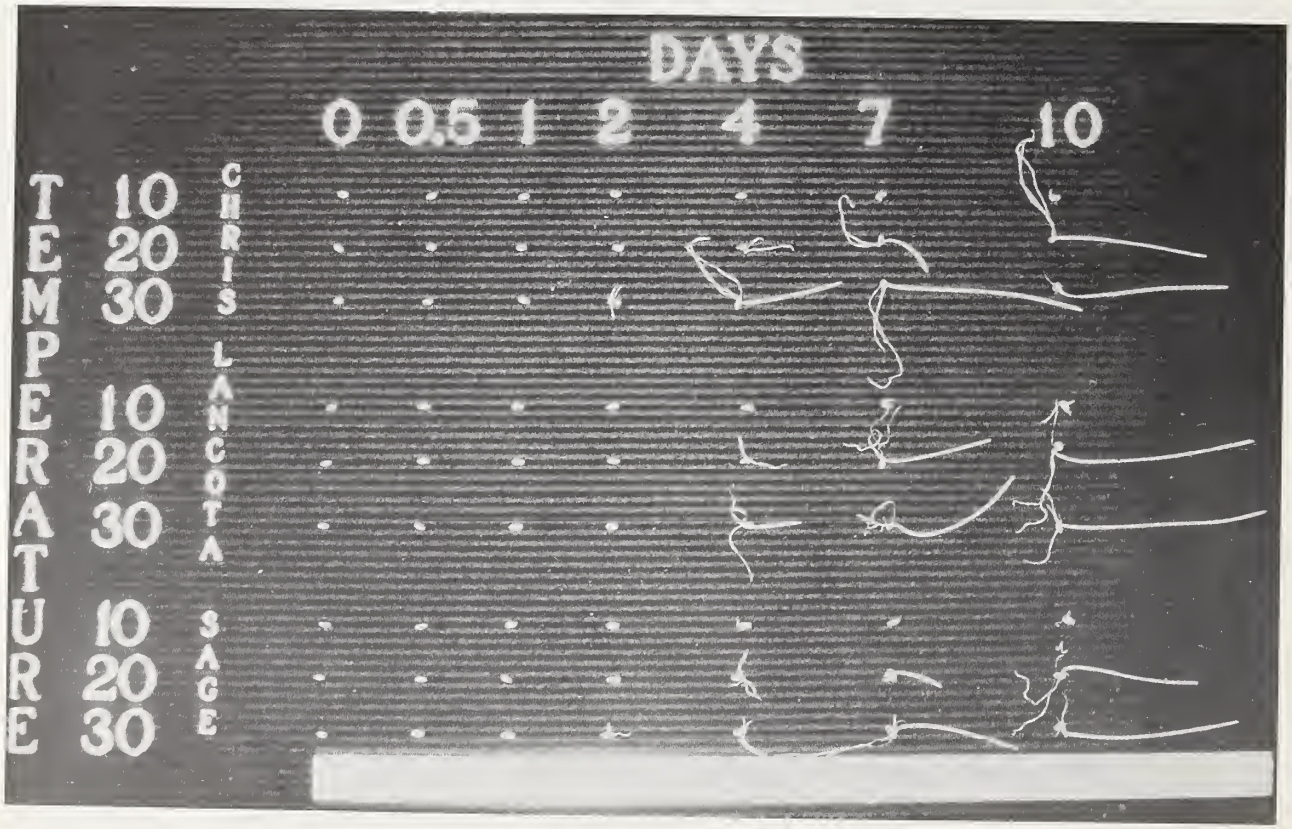

Figure 1. Appearance of 'Chris', 'Sage', and 'Lancota' wheat varieties germinated at three temperatures for seven different intervals.

Table 1. Sample number, variety, germination temperature, germination time, 500-seed weight, post-germination weight, and dry matter loss

Sample No.	Variety	Germination Temperature	Germination Time	500-Seed Weight	Post-Ger Weight	Dry Matter Loss
		C	Days	g	g	%
C-2	Chris	30	10	12.86	5.31	59
C-4	"	20	10	12.55	11.40	9
C-6	"	10	10	12.98	11.22	14
C-8	"	30	7	12.86	8.79	32
C-10	"	20	7	12.85	11.31	12
C-12	"	10	7	13.17	12.70	4
C-14	"	30	4	13.07	10.96	16
C-16	"	20	4	12.87	12.05	6
C-18	"	10	4	12.95	12.58	3
C-20	"	30	2	12.66	11.86	6
C-22	"	20	2	12.56	12.10	4
C-24	"	10	2	12.80	12.55	2
C-26	"	30	1	13.04	12.61	3
C-28	"	20	1	12.56	12.23	3
C-30	"	10	1	13.15	12.88	2
C-32	"	30	0.5	12.40	12.00	3
C-34	"	20	0.5	12.44	12.15	2
C-36	"	10	0.5	12.86	12.52	3
C-38	"	30	0	13.35	13.35	0
C-40	"	20	0	12.75	12.75	0
C-42	"	10	0	12.94	12.94	0
S-1	Sage	30	10	17.11	7.13	58
S-4	"	20	10	17.23	14.41	16
S-6	"	10	10	16.89	15.76	7
S-7	"	30	7	17.78	13.52	24
S-10	"	20	7	17.21	15.02	13
S-12	"	10	7	17.14	16.08	6
S-13	"	30	4	16.48	14.42	12
S-16	"	20	4	16.94	16.02	5
S-18	"	10	4	16.63	15.83	5
S-20	"	30	2	16.17	15.02	7
S-22	"	20	2	16.45	15.64	5
S-24	"	10	2	16.88	16.21	4
S-26	"	30	1	16.65	15.84	5
S-28	"	20	1	15.94	15.34	4
S-30	"	10	1	17.41	16.83	3
S-32	"	30	0.5	16.29	15.52	5
S-34	"	20	0.5	16.35	15.54	5
S-36	"	10	0.5	16.05	15.46	4
S-38	"	30	0	16.29	16.29	0
S-40	"	20	0	16.30	16.30	0
S-42	"	10	0	16.50	16.50	0

Table 1. (continued)

Sample No.	Variety	Germination Temperature	Germination Time	500-Seed Weight	Post-Ger Weight	Dry Matter Loss
		C	Days	g	g	%
L-1	Lancota	30	10	15.97	10.92	32
L-3	"	20	10	15.78	13.59	14
L-6	"	10	10	15.44	14.59	6
L-7	"	30	7	15.89	13.26	16
L-10	"	20	7	15.81	14.01	11
L-12	"	10	7	15.76	14.65	7
L-14	"	30	4	15.89	14.23	10
L-16	"	20	4	15.87	14.90	6
L-18	"	10	4	16.23	15.45	5
L-20	"	30	2	15.45	14.56	6
L-22	"	20	2	15.80	15.01	5
L-24	"	10	2	15.45	14.81	4
L-26	"	30	1	15.65	14.87	5
L-28	"	20	1	16.12	15.47	4
L-30	"	10	1	15.89	15.28	4
L-32	"	30	0.5	15.54	14.75	5
L-34	"	20	0.5	15.78	14.48	8
L-36	"	10	0.5	15.68	15.02	4
L-38	"	30	0	15.93	15.93	0
L-40	"	20	0	15.58	15.58	0
L-42	"	10	0	15.92	15.92	0

Table 2. Effect of germination on recovery of water-soluble and heat-stable water-soluble protein from three wheat varieties.

Variety*	Germination Temperature	Germination Time	Water-Soluble Protein	Heat-Stable Protein	
	C	Days	Mg/500 Seeds	Mg/500 Seeds	%
Chris	30	10	1311	1306	100
"	20	10	1218	1172	96
"	10	10	350	262	75
"	30	7	1078	956	89
"	20	7	1183	1142	97
"	10	7	287	229	80
"	30	4	1069	1041	97
"	20	4	1272	1213	95
"	10	4	277	266	96
"	30	2	806	833	100
"	20	2	409	375	92
"	10	2	504	442	88
"	30	1	428	410	96
"	20	1	324	322	99
"	10	1	266	282	100
"	30	0.5	213	160	75
"	20	0.5	206	153	74
"	10	0.5	209	161	77
"	--	0	211	167	79
"	--	0	215	176	82
Sage	30	10	1213	1201	99
"	20	10	885	866	98
"	10	10	282	229	81
"	30	7	1168	1166	100
"	20	7	1307	1213	93
"	10	7	231	229	99
"	30	4	1225	1202	98
"	20	4	509	442	87
"	10	4	319	287	90
"	30	2	492	493	100
"	20	2	271	234	86
"	10	2	352	264	75
"	30	1	319	255	80
"	20	1	369	266	72
"	10	1	285	245	86
"	30	0.5	302	220	73
"	20	0.5	183	139	76
"	10	0.5	271	229	85
"	--	0	211	157	75

Table 2, continues

Variety*	Germination Temperature	Germination Time	Water-Soluble Protein	Heat-Stable Protein	
	C	Days	Mg/500 Seeds	Mg/500 Seeds	%
Lancota	30	10	754	745	99
"	20	10	738	750	100
"	10	10	627	569	91
"	30	7	1032	1183	100
"	20	7	410	393	96
"	10	7	241	231	96
"	30	4	1061	1051	99
"	20	4	257	206	80
"	10	4	240	231	96
"	30	2	526	544	100
"	20	2	264	229	87
"	10	2	209	192	92
"	30	1	278	250	90
"	20	1	226	227	100
"	10	1	264	241	91
"	30	0.5	255	201	79
"	20	0.5	183	134	73
"	10	0.5	200	169	86
"	--	0	213	150	71
"	--	0	220	167	76

*Average weight per 500 seeds was 12.9 g for Chris, 16.7 g for Sage, and 15.7 g for Lancota.

Table 3. Change in amino acid composition of 'Sage' wheat germinated at 20 C for different times

Component	Sample Number						
	S-4	S-10	S-16	S-22	S-28	S-34	S-40
Days Germination	10	7	4	2	1	0.5	0
N (%)	2.58	2.54	2.34	2.37	2.34	2.36	2.26
N x 5.7	14.7	14.5	13.3	13.5	13.3	13.4	12.9
Amino Acids	----------g/16 g N----------						
alanine	4.2	3.7	3.7	3.4	3.5	3.4	3.8
valine	4.7	4.3	4.8	4.5	4.5	4.5	5.2
glycine	4.0	3.7	4.0	3.9	4.0	3.9	4.5
isoleucine	4.0	3.5	4.1	3.8	3.8	3.8	4.1
leucine	6.3	6.2	6.9	6.8	6.8	6.7	7.6
proline	7.7	8.7	10.1	10.2	10.1	10.0	11.3
threonine	2.6	2.5	2.7	2.6	2.7	2.6	3.0
serine	3.4	3.7	4.2	4.1	4.2	3.9	4.5
methionine	1.5	1.4	1.5	1.4	1.4	1.3	1.4
phenylalanine	8.7	4.3	4.8	4.7	4.7	4.7	5.3
aspartic acid	9.0	6.8	5.3	4.8	4.9	4.8	5.5
glutamic acid	21.6	24.9	29.4	31.0	30.6	30.5	33.7
tyrosine	2.7	2.6	2.6	2.3	2.2	2.2	2.1
lysine	3.65	3.1	2.9	2.6	2.7	2.6	2.8

Table 4. Change in amino acid composition of 'Sage' wheat germinated at 30 C for different times

Component	Sample Number						
	S-1	S-7	S-13	S-20	S-26	S-32	S-38
Days Germination	10	7	4	2	1	0.5	0
N (%)	2.68	2.83	2.46	2.39	2.32	2.38	2.29
N x 5.7	15.3	16.1	14.0	13.6	13.2	13.6	13.0
Amino Acids	----------g/16 g N----------						
alanine	5.1	4.7	3.9	3.5	3.3	3.3	3.3
valine	4.9	4.9	4.6	4.5	4.3	4.4	4.4
glycine	4.1	4.0	4.0	4.1	3.9	3.9	4.0
isoleucine	3.8	3.7	3.7	3.7	3.6	3.9	3.8
leucine	5.8	5.9	6.6	6.6	6.5	6.6	6.5
proline	5.1	6.1	8.9	9.6	10.0	10.1	10.1
threonine	2.8	2.8	2.7	2.5	2.6	2.4	2.6
serine	3.0	3.1	3.6	3.9	4.2	4.0	4.3
methionine	1.3	1.3	1.4	1.3	1.3	1.3	1.3
phenylalanine	3.8	3.9	4.5	4.4	4.6	4.6	4.6
aspartic acid	15.2	13.9	6.4	4.8	4.7	4.5	4.8
glutamic acid	14.9	17.0	26.4	28.9	30.0	29.8	30.1
tyrosine	1.3	1.4	1.9	1.8	1.6	1.9	2.1
lysine	4.2	3.7	2.9	2.7	2.6	2.6	2.6

Table 5. Change in amino acid composition of 'Lancota' wheat germinated at 30 C for different times

Component	Sample Number						
	L-1	L-7	L-14	L-20	L-26	L-32	L-38
Days Germination	10	7	4	2	1	0.5	0
N (%)	2.91	2.74	2.55	2.58	2.49	2.55	2.52
N x 5.7	16.6	15.6	14.6	14.7	14.2	14.5	14.4
Amino Acids	----------g/16 g N----------						
alanine	4.7	3.5	3.6	3.2	3.1	3.1	3.2
valine	4.7	4.4	4.6	4.2	4.2	4.3	4.3
glycine	3.9	3.9	4.1	3.8	3.7	3.9	3.9
isoleucine	3.3	3.6	3.8	3.3	3.4	3.5	3.5
leucine	5.7	6.2	6.6	6.3	6.2	6.5	6.3
proline	6.9	9.6	10.1	10.3	10.4	10.5	10.7
threonine	2.7	2.5	2.6	2.4	2.2	2.5	2.4
serine	3.4	3.7	3.9	3.8	3.7	4.0	4.0
methionine	1.1	1.2	1.2	1.1	1.2	1.2	1.2
phenylalanine	3.9	4.3	4.5	4.3	4.2	4.4	4.3
aspartic acid	14.1	5.6	5.5	4.1	4.0	4.4	4.4
glutamic acid	19.2	27.2	28.7	29.0	28.9	30.3	29.5
tyrosine	1.9	2.4	2.4	2.2	2.1	2.1	1.7
lysine	3.6	2.7	2.8	2.3	2.3	2.4	2.4
tryptophan	1.74	1.53	1.35	1.43			1.39

Table 6. Change in dry weight, total protein, and total lysine of 'Sage' and 'Lancota' wheats germinated for different times (data not corrected for moisture)

Germination Time	Dry Weight	Total Protein	Total Lysine
days	----------g/500 seeds----------		mg/500 seeds
	Sage - 20 C		
0	16.30	2.10	65.20
0.5	15.54	2.08	60.61
1	15.34	2.04	61.36
2	15.64	2.11	61.00
4	16.02	2.13	67.28
7	15.02	2.18	73.60
10	14.41	2.12	85.02
	Sage - 30 C		
0	16.29	2.12	58.64
0.5	15.52	2.11	57.42
1	15.84	2.09	60.19
2	15.02	2.04	60.08
4	14.42	2.02	63.45
7	13.52	2.18	87.88
10	7.13	1.09	50.62
	Lancota - 30 C		
0	15.93	2.29	60.53
0.5	14.75	2.14	56.05
1	14.87	2.11	53.53
2	14.56	2.14	53.87
4	14.23	2.08	64.04
7	13.26	2.07	61.00
10	10.92	1.81	72.07

THE ROLE OF CEREAL FIBER IN HUMAN NUTRITION AND HEALTH; GLUCOSE TOLERANCE AND DIABETES

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Specific changes in the pattern of carbohydrate consumption are characteristic of societies as they become urbanized or developed. In general, the consumption of foods containing complex carbohydrates and fiber decreases while the consumption of foods rich in simple sugars increases. In the United States the average per capita consumption of flours and cereals has dropped from 300 pounds/year in 1909 to 141 pounds/year in 1970. The same period has witnessed an increase in the per capita consumption of refined sugar and other sweeteners from 87 to 126 pounds/year.

Dietary fiber has long been neglected as an important factor in human nutrition, since it is a negligible source of energy and no specific deficiency symptoms develop in its absence. Recently, epidemiological observations coupled to an increasing knowledge of the physiological role of dietary fiber at the intestinal level have linked a variety of diseases prevalent in western civilizations and virtually absent in underdeveloped societies to a relative deficiency in dietary fiber (4, 23). The daily intake of crude fiber in the United States and Great Britain has been estimated to be in the range of 4 to 12 g (5, 6, 22) or 16 to 25% the consumption in these countries 100 years ago (18, 22). In contrast, the fiber intake by rural South African Bantu tribesmen is approximately 25 g per day (14). In this context, the intake of fiber in excess of that currently consumed in western civilizations may produce decreases in the prevalence of noninfective, degenerative diseases characteristic of these societies.

Diabetes and heart disease represent two of the most critical health problems in the United States today. Diabetes now affects about 5 million Americans and results in over 35,000 deaths annually. Diabetes is a primary cause of blindness in the United States. A close relationship between diabetes and heart disease is indicated by the finding that diabetics have a much higher risk of developing heart disease than the general population (12) and that 25% or more of patients with cardiovascular disease show abnormal glucose tolerance (24).

It has been proposed that the removal of fiber from foods is indirectly responsible for the increased incidence of diabetes by promoting overconsumption and obesity (4, 7). Obesity is generally considered to be an important contributory factor in the causation of diabetes and heart disease. Recently, a number of studies have appeared in the literature that appear to show a direct relationship between the consumption of dietary fiber and improvement of glucose tolerance and symptoms of clinical diabetes. It is the purpose of this review to describe these studies and to discuss the possible mechanisms involved.

The concurrent consumption of fiber during a glucose tolerance test has been found to lower glucose and insulin responses in normal and diabetic subjects. Table 1 presents the results of a study by Jefferys and Macdonald (9) in which six healthy subjects, aged 20 to 25 years, were given a glucose tolerance test consisting of 1 g glucose syrup without (control) and with 0.2 g/kg body weight of the indicated sources of fiber. The increases in blood glucose above fasting were determined after 30, 60, 90 and 120 minutes. Bran significantly ($P < 0.01$) improved glucose tolerance at 60, 90 and 120 minutes compared within subjects to the control. Bagasse and wood cellulose were without effect, indicating that not all sources of fiber influence glucose tolerance.

TABLE 1.--Effect of various sources of fiber on glucose tolerance test in young healthy humans

Time	Increase in blood glucose			
	Control	Bran	Bagasse	Wood cellulose
min.		mg/100 ml		
30	46 ± 6	37 ± 5	66 ± 8	59 ± 4
60	43 ± 8	26 ± 4	59 ± 12	61 ± 8
90	28 ± 4	13 ± 3	24 ± 5	16 ± 6
120	16 ± 2	6 ± 3	13 ± 3	18 ± 5

Values represent the mean ± SEM from 6 subjects.

Glucose tolerance mixture = 1 g glucose syrup + 0.2 g fiber/kg body weight.

Adapted from Reference No. 9.

In a similar type of study, Jenkens and coworkers (10) fed diabetic volunteers one of two test meals after an overnight fast. The meals differed only in that one contained 16 g guar powder in bread and 10 g pectin in marmalade. Figures 1 and 2 show the effect on blood glucose and insulin, respectively, as a function of time after meal. After the meal containing guar and pectin, the mean blood glucose level was significantly below the control at 30, 45, 60 and 90 minutes. Similarly, serum insulin levels were significantly lower 30 to 120 minutes after the fiber-containing meal. A subsequent study (11) has shown a 46% decrease in mean urinary glucose excretion by diabetics consuming 25 g guar gum daily for 5 or 7 days.

In a study in which the type of fiber employed was not defined, eight insulin-requiring diabetics were placed on identical diets differing only in the level of fiber (3 or 20 g crude/day) for 10 days in random order (15). Table 2 shows the plasma glucose levels at four different times during the day on 3 separate days. Except after fasting, the levels of glucose were significantly less during the intake of the high fiber diet.

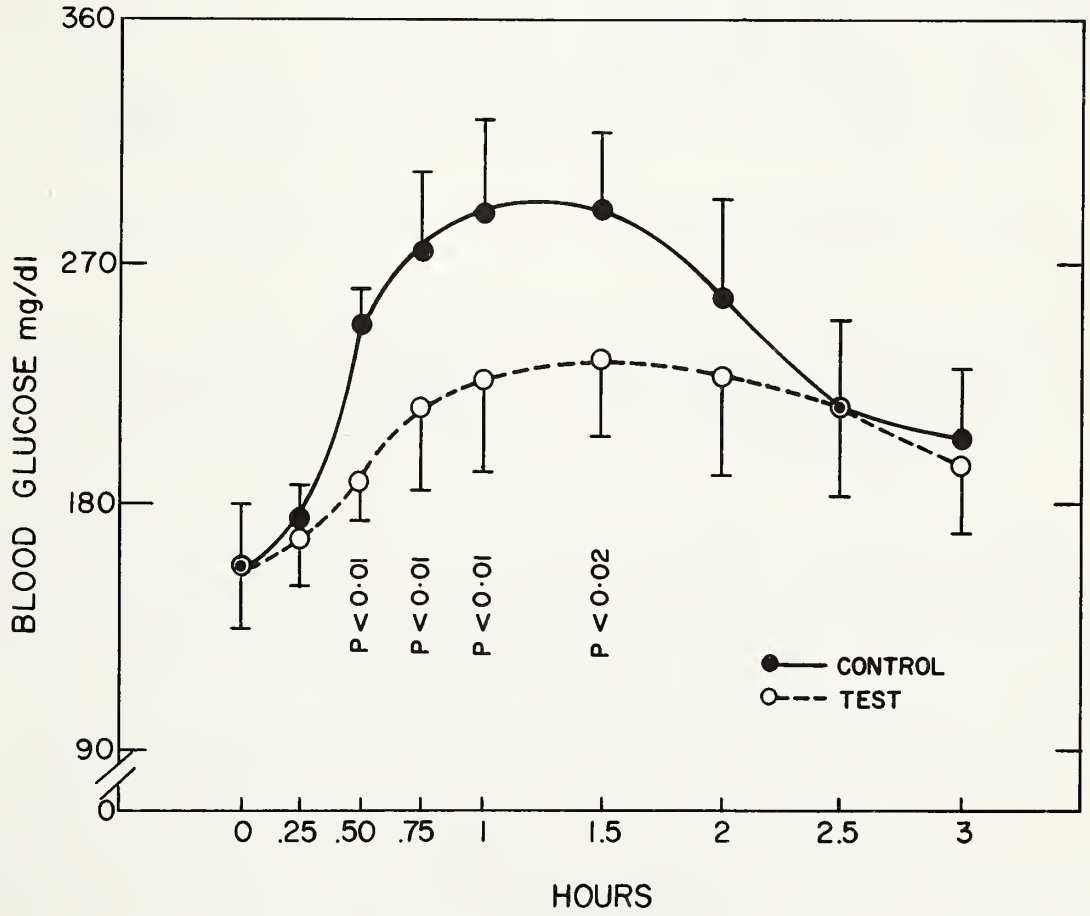

Figure 1.--Mean blood glucose levels (mg/dl) ± SEM of 8 non-insulin-requiring diabetics after taking control and fiber-enriched test meals.

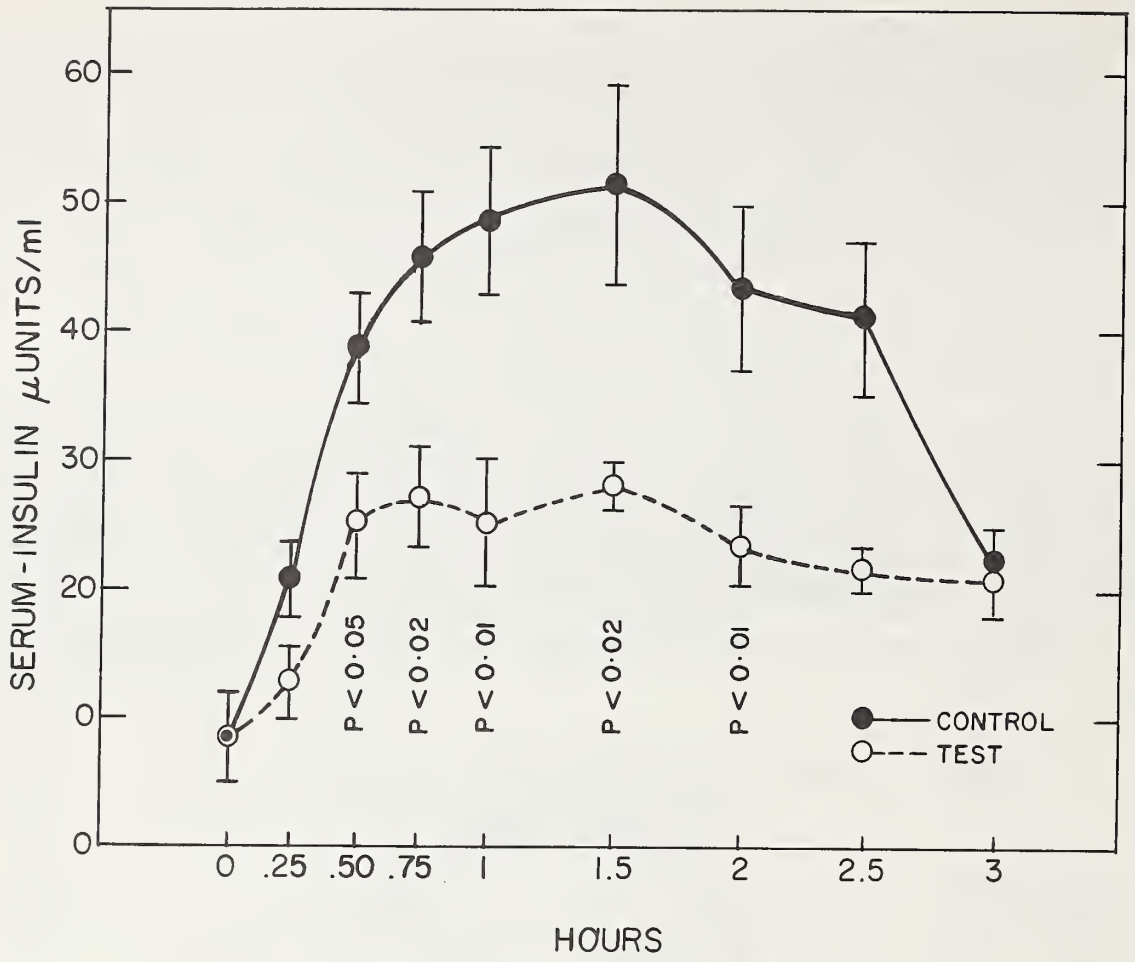

Figure 2.--Mean blood insulin levels (μunits/ml) ± SEM of 8 non-insulin-requiring diabetics after taking control and fiber-enriched test meals.

The physiological effects of dietary fiber on parameters of glucose tolerance are believed to be manifested at the intestinal level, since fiber is not appreciably digested or absorbed. The presence of 16 to 32 g of fiber from fruits, vegetables and wholemeal bread has been shown to increase the fecal loss of utilizable compounds of the diet (19). This suggests that fiber may decrease the bioavailability of utilizable carbohydrate, perhaps by limiting access to the absorptive mucosal surface. The decreased availability of components of the carbohydrate diet or glucose of a test meal could prevent the steep increases in postprandial blood glucose and the associated strong stimulation of the insulin response associated with the ingestion of diets high in simple carbohydrates. The effect of fiber on blood glucose and insulin levels would be expected to be greater in onset diabetics than in normal individuals.

TABLE 2.--Effect of dietary fiber on plasma glucose levels in diabetics during 10-day periods

Diet	Blood glucose				
	Fasting	11:30 AM	5:00 PM	9:00 PM	Mean
		mg/100 ml			
Low fiber (3 g crude/day)		164	154	204	169 ± 12
	N.S.	S	S	S	P < 0.001
High fiber (20 g crude/day)		122	100	113	121 ± 10

Values represent the mean ± SEM from 8 diabetics obtained on 3 separate days during each dietary period.

Adapted from Reference No. 15.

The mammalian small intestine has apparently evolved a receptor system that reacts to glucose ingested orally by secreting an intestinal hormone (e.g., gastric inhibitory polypeptide (GIP)) that stimulates secretion of insulin in the pancreas. Improper functioning of these hormones may contribute to the pathogenesis of diabetes. It has been reported that glucose absorbed in the proximal intestine is a more efficient secretagogue for GIP than is glucose absorbed more distally in the intestine (21). Since fiber would be expected to decrease the availability of glucose for absorption at the proximal intestine, a role of fiber in glucose tolerance by an effect on gastrointestinal hormones is suggested. Glucagon and other hormones involved in glucose homeostasis may similarly be influenced by intestinal events modulated by dietary fiber.

Dietary fiber does not have to be consumed concurrently with a meal in order to effect parameters of glucose tolerance. Indirect evidence for this comes from studies in which Bantu schoolchildren (25) and African cleaners (26) showed significantly lower blood glucose levels following a fasting 50 g glucose tolerance than did Caucasian schoolchildren (25) and Europeans (26), respectively. These studies suggest that humans adapted to diets high in fiber show improved glucose tolerance. More direct evidence for this contention comes from a study by Rodger and coworkers (17) (Table 3).

TABLE 3.--Plasma insulin response after oral glucose tolerance tests in hypertriglyceridemic, mildly diabetic men fed diets containing 30-38% of calories as either simple or complex carbohydrates

Diet	Plasma insulin		
	90 minutes	120 minutes	Summed increments (30-120 minutes)
		μunits/ml	
Initial	143 ± 12	157 ± 19	399 ± 50
Simple carbohydrate	125 ± 10	157 ± 31	362 ± 75
Complex carbohydrate	74 ± 13*	87 ± 21*	205 ± 46*

Values represent the mean ± SEM from 5 subjects.

* Significantly different ($P < 0.01$) from responses on initial and simple carbohydrate diets.

Adapted from Reference No. 17.

Five hypertriglyceridemic, mildly diabetic men were fed diets in which 75% of the carbohydrate was supplied by either foods containing simple sugars (e.g., sucrose, fructose, lactose) or by foods containing complex carbohydrate (e.g., starch and fiber) in a crossover design. No changes in body weight occurred during the 3- to 5-week dietary periods. A glucose tolerance was performed following an overnight fast prior to and after the dietary periods. Plasma insulin 90 and 120 minutes after the tolerance as well as the summed insulin increments 30 to 120 minutes after the tolerance were all significantly lower after the period in which complex carbohydrate was consumed. Blood glucose levels determined concurrently were not effected by the nature of the dietary carbohydrate. It can therefore be concluded that the subjects were more insulin sensitive after adaptation to the high fiber diet.

In another study, 37 patients with diverticular disease were given 24 g of Prewett's bran daily for at least 6 months (3). There was no significant change in body weight during this time. Oral glucose tolerance after a 12-hour fast was compared in the same subject before and after the dietary period (Figure 3). Blood glucose peaks were significantly lower at 60 and 90 minutes after consuming the bran. The decreased response was attributed to a delayed intestinal absorption of glucose.

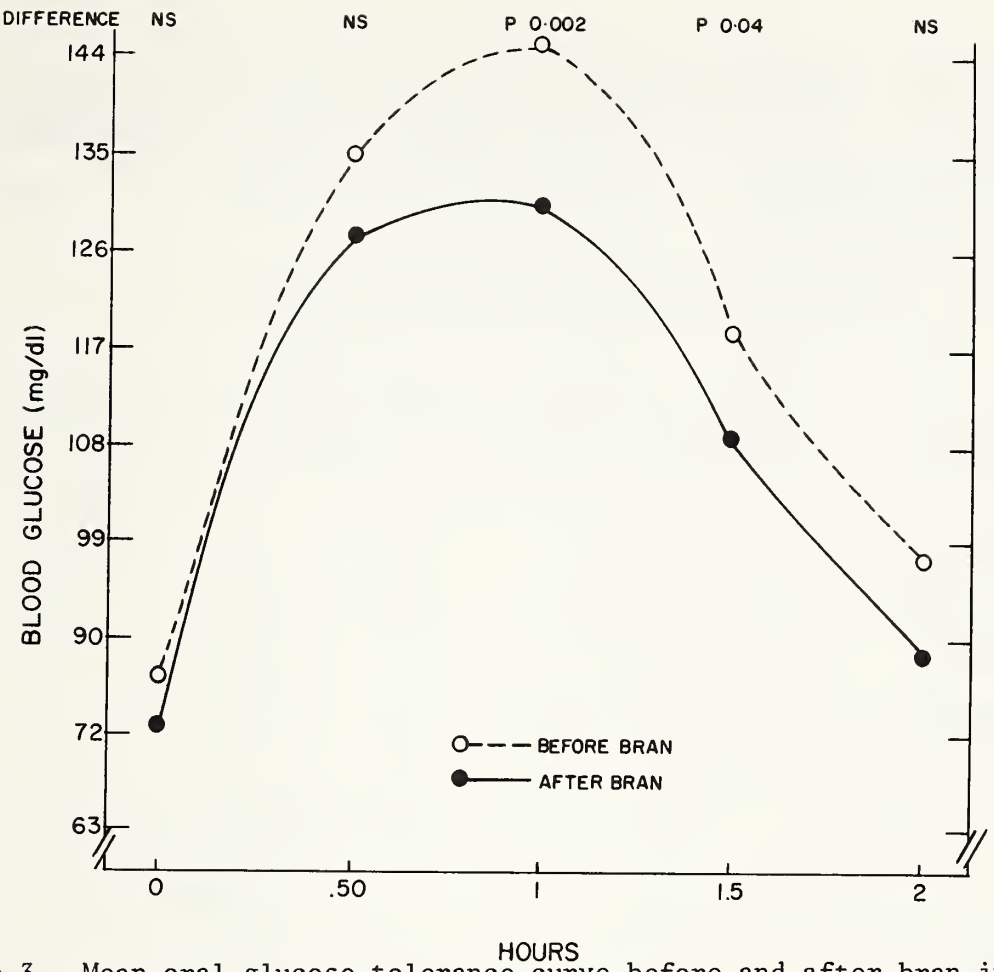

Figure 3.--Mean oral glucose tolerance curve before and after bran in 37 subjects.

Evidence linking levels of circulating insulin with the pathogenesis of atherosclerosis has been reported (20). It is therefore possible to attribute the increased rate of heart disease in diabetics to the oral hypoglycemic drugs and insulin used to treat the disease. The desirability of employing means for treating diabetes without producing transient and nonphysiological increases in circulating insulin levels is thus apparent. The use of diets high in fiber to treat and control diabetes is being successfully employed by Dr. James W. Anderson and his associates (1, 2, 13). Table 4 shows the composition of a standard diabetic diet containing 43% calories as carbohydrate and a high-carbohydrate (75%), high-fiber (71 g dietary fiber/day) used by Anderson to treat diabetics. The high-carbohydrate, high-fiber diet contained less fat, cholesterol and protein than the American Diabetic Association (ADA) diet. The increased amount of carbohydrate is in the form of complex carbohydrate, with simple carbohydrate (sugars) contributing the same percent of calories in both diets. The high

carbohydrate diet supplies only about 10 g of sucrose/day or less than 2% of the calories. The high-carbohydrate diet thus conforms to the dietary recommendations made by the U.S. Select Committee on Nutrition and Human Needs (e.g., less saturated fat, cholesterol, sucrose; more complex carbohydrate and fiber).

TABLE 4.--Composition of 2200 Kcal diets

Component	American Diabetic Association		High-Carbohydrate	
	g	% cal	g	% cal
Total carbohydrate	234	43	414	75
Simple	109	20	112	20
Complex	125	23	302	55
Protein	128	23	86	16
Fat	83	34	23	9
Cholesterol	0.54	-	0.06	-
Crude fiber	4.6	-	20.9	-
Dietary fiber	10.0	-	70.6	-

Adapted from Reference No. 2.

Table 5 gives the source of the dietary fiber in both the ADA and high-carbohydrate diets. The principal source of fiber in the high-carbohydrate diet is from grain products, principally wheat bran.

A comparison of fasting blood glucose, cholesterol and triglyceride levels in diabetic men fed either the ADA or high-carbohydrate, high-fiber diet for 2 weeks is shown in Table 6. The diets were designed to maintain all patients at the same weight level throughout the study. Plasma glucose levels were reduced about 33% ($P < 0.001$) when the subjects consumed the high-carbohydrate diet. The decrease in serum cholesterol on the high-carbohydrate diet is probably due to the reduced intake of dietary

TABLE 5.--Fiber content of 2200 Kcal diets

Food	American Diabetic Association		High-Carbohydrate		
	Crude	Dietary	Crude	Dietary	%
	g/day	g/day	g/day	g/day	
Grain products*	0.3	0.8	9.4	37.6	53
Lima beans	-	-	2.2	6.6	9
Brown rice and sweet potatoes	0.7	1.2	2.3	8.1	12
Vegetables	1.6	3.8	3.8	9.8	14
Fruits	2.0	4.2	3.2	8.5	12
Totals	4.6	10.0	20.9	70.6	100

* Principal source of grain fiber is wheat bran.

Adapted from Reference No. 2.

cholesterol. Diets high in carbohydrate, especially sucrose, are known to increase fasting levels of blood triglyceride (16). The decrease in fasting serum triglyceride on the high-carbohydrate diet is probably due to a combination of the low level of dietary sucrose and the high level of fiber which has been reported to lower serum triglyceride in humans (8). These results show that the high-fiber diet reduces the levels of three risk factors associated with either heart disease or diabetes.

Table 7 shows the results of the treatment of 37 diabetic patients with the high-carbohydrate diet since September 1976. On the basis of fasting blood glucose levels and response to a glucose tolerance test, the high-carbohydrate, high-fiber diet appears to be very effective in treating patients requiring less than 20 units of insulin per day or those treated with oral hypoglycemic agents. Satisfactory plasma glucose levels have been maintained after stopping oral agents and insulin therapy. These diets also produced a reduction in serum cholesterol and triglyceride levels. It, therefore, appears that modification of the U.S. diet as recommended by the Select Committee on Nutrition and Human Needs may be of benefit in preventing not only the chemical and clinical manifestations of diabetes but also the long term vascular complications associated with this disease.

TABLE 6.--Comparison of blood glucose, cholesterol and triglyceride values in diabetic men fed either the American Diabetic Association diet or a high-carbohydrate, high-fiber diet for 2 weeks

Diet	Plasma glucose	Serum cholesterol	Serum triglyceride
		mg/100 ml	
American Diabetic Association	179 ± 10	203 ± 13	180 ± 30
High-carbohydrate (75%) high-fiber	119 ± 7*	153 ± 9*	144 ± 23

Values represent the mean ± SEM from 10 subjects.

* Significantly lower ($P < 0.001$) than values from subjects fed the ADA diet.

Adapted from Reference No. 13.

TABLE 7.--Response of diabetic patients to the high (75%) carbohydrate, high fiber diet

Severity of diabetes	Number	Response		
		Complete	Partial	None
Chemical diabetes	14	13	0	1
Oral drugs	6	6	0	0
Insulin dependent				
15-20 Units/day	9	9	0	0
21-30 Units/day	3	0	3	0
> 30 Units/day	5	0	(1)	5

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THE BIOLOGICAL AVAILABILITY TO RATS OF IRON AND ZINC IN LOW-PHYTATE WHEAT BRAN

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Iron was the nutrient most consistently found to be lower than the Recommended Dietary Allowance in U.S. diets. Low iron intake probably was the major factor that contributed to low hemoglobin values in the U.S. population according to recent HANES (1). Marginal zinc deficiency occurs in the United States (2) and a recent survey showed that the zinc intake in U.S. households may be lower than expected (3). Thus, knowledge about factors that influence bioavailability of trace elements in foods is important.

Foods prepared from wheat and other cereals are important sources of iron and zinc in U.S. diets, but cereals contain phytic acid, possibly as mixed salts of phytate (4). Since the early 1940's phytic acid has been considered to decrease the bioavailability of iron (5). However, monoferric phytate, isolated from wheat bran, was shown to be soluble and readily available to the rat in comparison to an insoluble ferric phytate more completely saturated with iron (6). Thus, the number of iron atoms complexed to the phytate is one determining factor in the iron-phytate interrelation. The bioavailability of zinc is also influenced by phytate (7,8). The zinc of wheat supported growth of rats less effectively than zinc in foods of animal origin (9). Fiber also was implicated as a factor that reduces the bioavailability of iron and zinc in cereals (8,10).

Our study had two ojectives; (a) to produce phytate-free or low-phytate wheat bran preparations and (b) to determine the bioavailability to the rat of the iron and zinc in those preparations.

Methods

Preparation of low-phytate bran. An enzymatic and a non-enzymatic procedure were used to prepare low-phytate or phytate-free bran preparations.

Enzymatic procedure: wheat bran was suspended in 10 volumes of distilled water and incubated with shaking at 37°C overnight (16-20 hr.). The entire incubation suspension was freeze-dried and fed.

Non-enzymatic procedure: hard wheat bran was autoclaved 1 hr. at 15 psi, cooled and then extracted overnight at 37°C with 0.1M acetate buffer, pH 4.5. The suspension was allowed to settle, soluble material was decanted and discarded, the solids were washed with demineralized water and freeze-dried.

Zinc phytate preparations. Eight-tenths g of $ZnSO_4 \cdot 7H_2O$, dissolved in dilute acetic acid, was added slowly and with stirring to a solution of 8.5 g of Naphytate (product was about 50% phytic acid equivalent) in ammonium acetate. Ethanol was added to a final concentration of 50% by volume. A white precipitate formed and was collected, washed with 50% ethanol and dried. The precipitate contained 2.8 moles zinc/mole of phytate and will be referred to as Zn_3phytate. This preparation gave a cloudy suspension in water and could be eluted from P-4 Bio-Gel filtration medium.

Fifteen g of $ZnSO_4 \cdot 7H_2O$, dissolved in acetate buffer, was added slowly and with stirring to 6.25 g Naphytate dissolved in demineralized water. The resultant precipitate was washed with water and dried to give a water-insoluble product containing about 6 moles of zinc/mole of phytate. This will be refered to as Zn_6phytate.

Animal trials. A depletion-repletion hemoglobin assay was used for iron bioavailability (6). The low-iron basal diet (Table 1) was fed for 3 weeks to weanling, male rats[1]. The repletion diets were fed for 2 weeks, then hemoglobin levels of rats were determined in the blood obtained from the tail tip. Relative biological value was calculated by slope ratio. The reference compound was $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$.

The effect of the phytate/Zn ratio on bioavailability of zinc was assayed by growth and bone zinc of rats. Weanling rats were fed the respective diets ad libitum. They were caged individually and provided distilled water. Most of the zinc trials were of 4 weeks duration. Data were treated statistically by either Student's t test or analysis of variance and each datum is the average from 8 or 10 rats.

Analytical procedures. Metal analyses were by atomic absorption measurements on 1N HCl solutions of the ash from dry-ashed bran or diet (11). Phytate was extracted in dilute HCl, precipitated as the ferric salt, incubated in 0.5N HCl (if required), washed, dissolved in concentrated HCl and phosphate was determined on a wet-ashed aliquot for calculation as phytic acid (12). Dietary fiber analyses were by neutral detergent with an enzymatic step to eliminate starch (13).

Results

Solubility of iron, zinc and phytate. Solubility studies were conducted to gain some indication of the nature of the phytate, iron and zinc in wheat bran. Exhaustive extraction of wheat bran by 1.2M NH_4acetate in the cold extracted about 75% of the iron, 50% of the

zinc and 80% of the phytate. When this extract was chromatographed on Bio Gel P-4 gel filtration medium, the iron eluted as monoferric phytate, just before the major phytate peak as reported (6). The zinc eluted with the phytate, thus indicating that 50% of the zinc in wheat bran may exist as a zinc phytate complex that is extractable in aqueous 1.2M NH_4acetate. During extraction overnight in distilled water at 3° solubilities of iron, zinc and phytate in bran were, respectively, less than 5%, about 5%, and not detectable.

After incubation at 37°C overnight of a suspension of bran in water (1 g:10 ml), 100% of the phosphorus in the bran was soluble in the supernatant as inorganic phosphate and no monoferric phytate or zinc phytate was present. Endogenous phytase probably hydrolyzes the phytate under this condition. Antibiotics in the suspension did not prevent hydrolysis of the phytate indicating that microbiol action was not likely responsible. Iron was present as a soluble complex of undetermined nature, and in some preparations, was 100% soluble. When the supernatant was chromatographed, the iron eluted over a wide range of fractions. For some brans essentially all iron was in the reduced state, but for others only about 25% was reduced. We speculate that proteolytic activity might have been sufficient to provide free amino acids to form soluble iron-amino acid complexes. About 90% of the zinc remained in solution and chromatographed as low molecular-weight or ionic species.

We autoclaved bran for 1 hour to inactivate the endogeous phytase; shorter time might have sufficed, but we did not attempt to establish the minimum conditions. Autoclaving rendered water soluble a slight amount of phytate but no additional iron or zinc. Aqueous 0.1M acetate buffer, pH 4.5, extracted about 90% of the phytate, but only small amounts of iron and zinc. Analyses showed (dry basis) for the residue of the autoclaved, acetate-extracted bran, 78.3 ppm zinc, 0.36% phytic acid, and 42.8% neutral detergent fiber; and for the initial bran, respectively, 90.1, 3.63% and 34.7%. The iron, zinc and phytate that remained in the autoclaved, 0.1M acetate-extracted bran residue were extracted by 1.2M NH_4acetate. When chromatographed on Bio-Gel P-4 the iron and zinc were present as the phytate complexes.

Bioavailability of iron in phytate free bran preparation. We reported (14) that the iron of wheat and its milling fractions was of good bioavailability to the rat; the relative biological value (RBV) was about 90 compared to $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ as 100. The RBV of four leavened whole wheat breads, however, averaged only 75, and for three of the breads ranged from 53 to 67 (14). Leavened bread contains less phytate than does unleavened (15, 16). During bread making the iron of monoferric phytate might be released as ferric iron upon hydrolysis of the phytate. Then ferric hydroxide might be formed and that iron would have a very low bioavailability[2]. Iron in the supernatant from bran that had been incubated in water at 37° overnight was predominantly ferrous and possibly complexed with amino

acids, but we tested its bioavailability to rats under conditions that were similar to conditions in the tests of bread (14). When the freeze-dried enzymatically prepared bran used for this trial was resuspended in demineralized water, the iron was not all soluble and about 60% was in the ferric state. We did not further pursue the chemical nature of the iron. The preparation was essentially free of phytate; no phytate was detected by the iron precipitation method and only a trace of phosphorus was present in the usual fraction for phytate in the gel filtration chromatogram. This freeze-dried bran and the raw bran were incorporated into test diets (Table 1) to provide 8, 16 and 24 ppm iron and bioassayed against $Fe(NH_4)_2(SO_4)_2$.

Table 1. Basal Diets for Rat Feeding Trials

Ingredient[1]	Fe basal	Zn basal
		g per kg
Vitamin free casein	200	-
Spray dried egg white solids	-	200
Glucose monohydrate[2]	700	680
Salts[3]	40	40
Non-nutritive fiber	-	20
Vitamin mix	10[4]	10[4]
Corn oil	50	50

[1]Corn oil was purchased at a local grocery, egg white from Henningsen Foods Inc., White Plains, NY and other ingredients from Teklad Test Diets, ARS Sprague Dawley, Madison, WI.

[2]Premixes and food iron or zinc source and other additions were added at the expense of glucose.

[3]Briggs, G.M. and Williams, M.A. (1963) Fed. Proc. 22: 261. Iron or zinc salt omitted for respective basal.

[4]Additional 2 mg/kg of biotin was added to the Zn basal as glucose premix. Ascorbic acid was omitted from the commercial mix.

The RBV for the iron in the phytate-free bran was 113 with 95% confidence interval of 104 to 124 (Fig. 1). The RBV for iron in the raw bran was 98. As discussed above, possibly the iron was present as stable amino acid-iron complexes that would be readily available, particularly if a histidine-iron complex (17). Both types of bran provided equal amounts of dietary fiber to the diets.

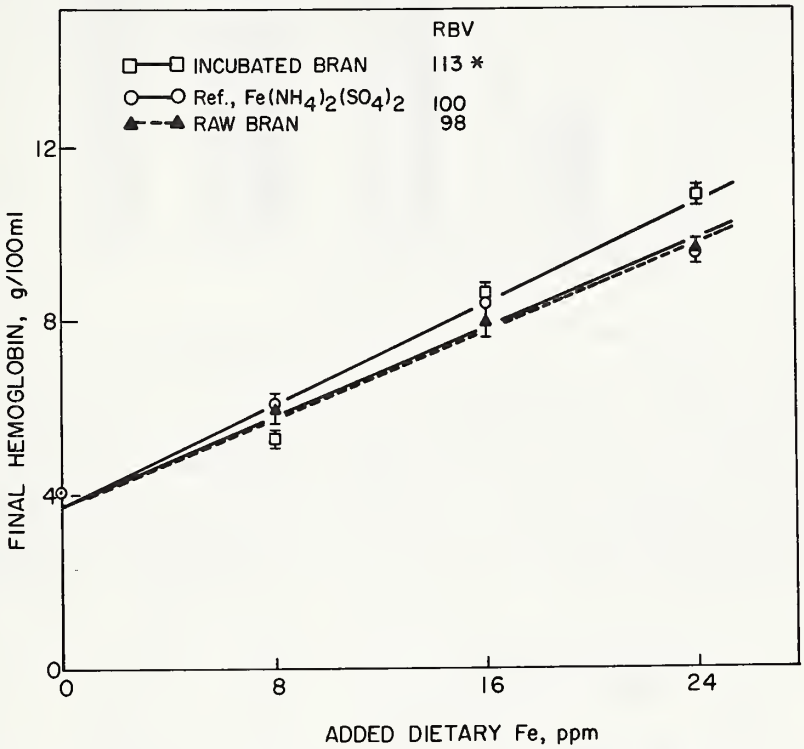

Figure 1. The Biological Availability of Iron in Phytate-Free Bran. Phytate-free bran was prepared by the enzymatic procedure and is designated incubated bran. Each data point is the average from 10 rats.

*95% confidence interval = 104 to 124.

Effect of the phytate/zinc ratio on zinc utilization. Effects were determined of the phytate/zinc molar ratio on the growth response of rats to $ZnSO_4 \cdot 7H_2O$ under the dietary conditions in our laboratory. One experiment tested the bioavailability of zinc from zinc phytate complexes. Both the Zn_3phytate and the Zn_6phytate were equivalent to the $ZnSO_4$ in supporting growth (Fig 2). The zinc of those preparations may dissociate from the phytate at the pH of the rat's stomach (18). Although some phytate probably remained, sufficient zinc was absorbed from the diets to support growth.

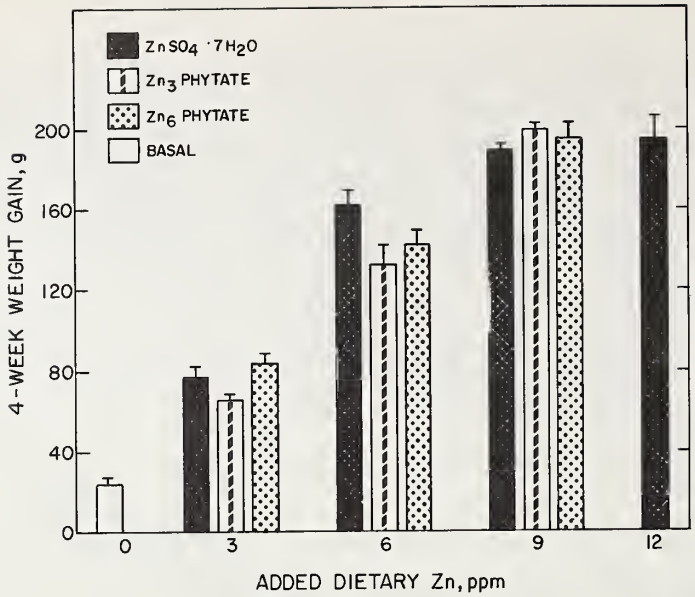

Figure 2. Growth Response of Rats to Zinc as Zn Phytate.

In a second phytate/zinc experiment, (Fig. 3) all diets contained 10 ppm zinc as $ZnSO_4$ and Naphytate was added to obtain the desired phytate/zinc ratio. With approximately 0.8% calcium in the diets (Williams-Briggs Salts w/o added calcium) growth was not affected by a ratio of 12, but was depressed by a ratio of 24. When extra calcium (1%) was added however, growth tended to be depressed at ratio 6, but the level of depression was not significant below that at ratio 9.

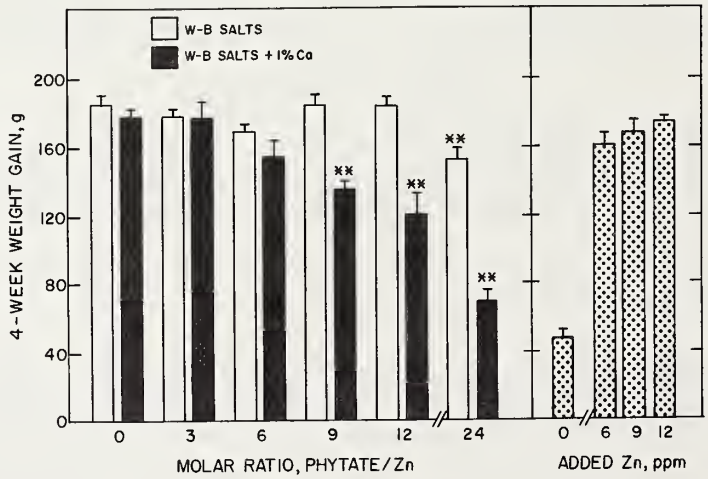

Figure 3. The Effect of Phytate/Zinc Ratio on Growth of Rats. Right side of figure shows growth response to graded levels of zinc as $ZnSO_4 \cdot 7H_2O$ with no phytate. All other diets contained 10 ppm zinc and phytate was added as Naphytate.

**Gain significantly less than ratio 0, $P<0.01$.

Bioavailability of zinc in low-phytate bran. In Fig. 4 responses are compared among rats provided zinc in a low-phytate bran, $ZnSO_4$ and in raw bran. The low-phytate bran was prepared by the enzymatic procedure. The phytate content was reduced from 4.0% in the raw bran to 0.8% in the low-phytate bran. Growth response was significantly less ($P < 0.01$) for raw bran than for either the reference $ZnSO_4$ or the low-phytate bran. As zinc source, the low-phytate bran supported slightly lower growth at 6 ppm than did $ZnSO_4$, but at 12 ppm there was no difference between the two sources. The phytate/zinc ratios for the raw bran and the low-phytate bran were 46 and 8, respectively.

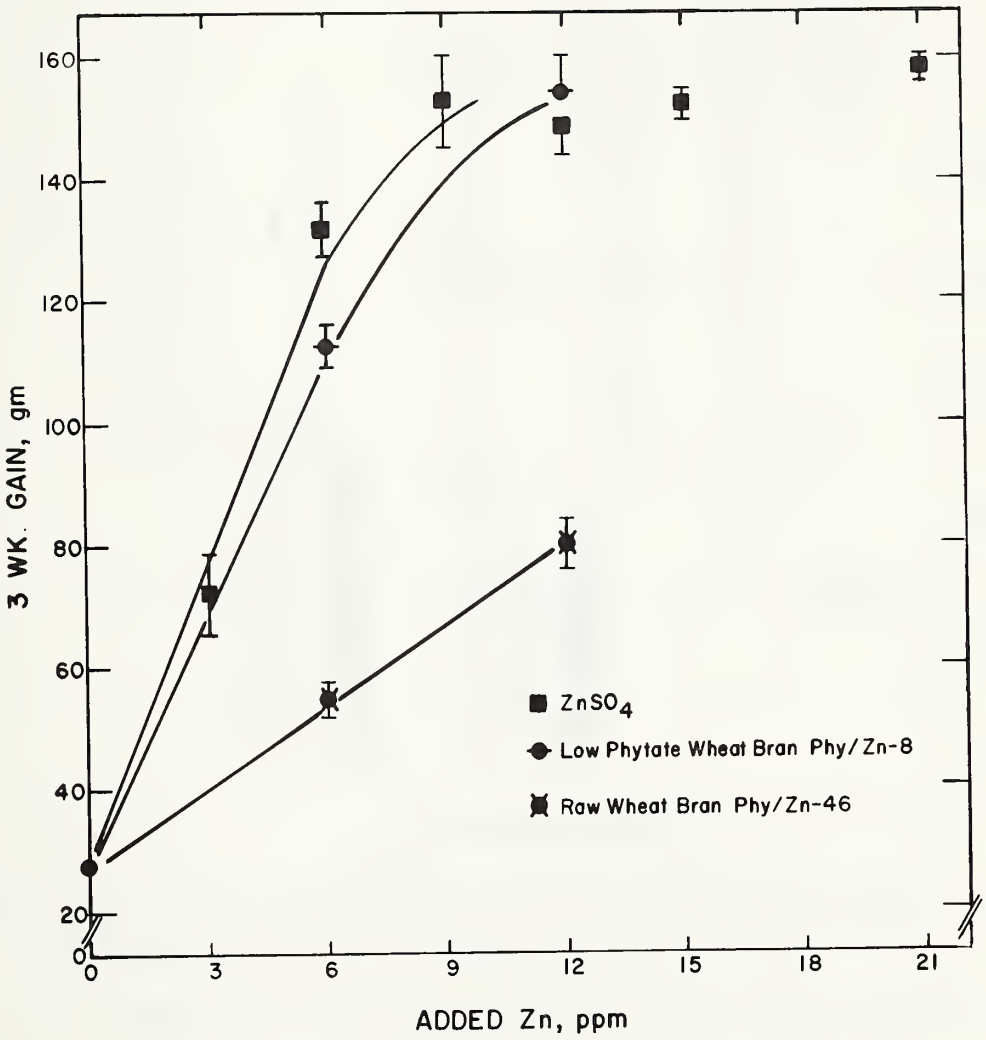

Figure 4. Low-Phytate Bran Produced by Enzymatic Procedure as Dietary Zinc Source for Rats.

Low phytate bran, which contained 0.36% phytic acid, was prepared by the non-enzymatic procedure and was used in a second zinc trial. A sufficient quantity of the bran was autoclaved so that subsamples of autoclaved bran and autoclaved bran that had been incubated with 0.1M acetate buffer and then freeze-dried could be included as comparative zinc sources. The phytate/zinc ratio of the raw, autoclaved, and incubated-autoclaved brans was 40 and of the low phytate bran, 4.5. Figures 5 and 6 present the results of this experiment.

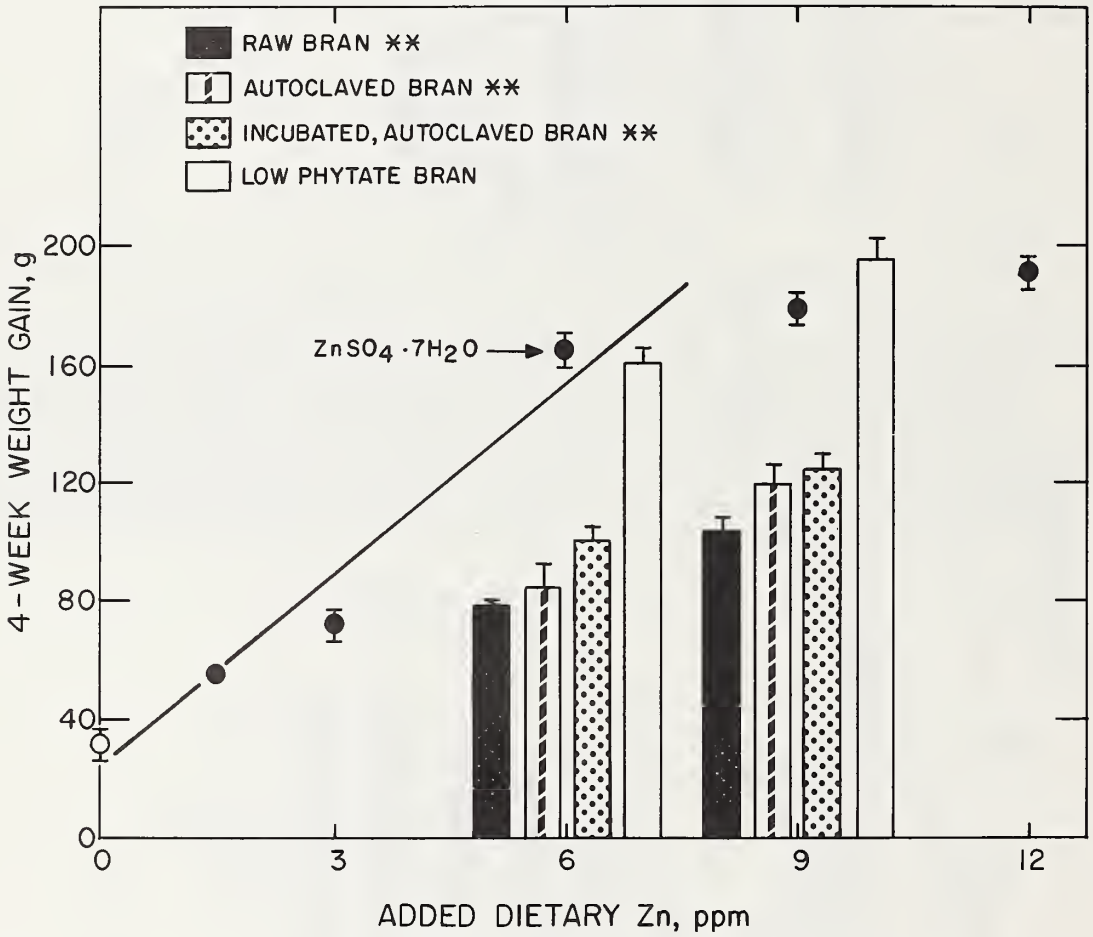

Figure 5. Growth Response of Rats to Low-Phytate Wheat Bran as Zinc Source. Low-phytate bran was produced by non-enzymatic procedure. The brans were incorporated in diets to supply 6 and 9 ppm dietary zinc.

**Growth response significantly different from that for $ZnSO_4$, $P<0.01$.

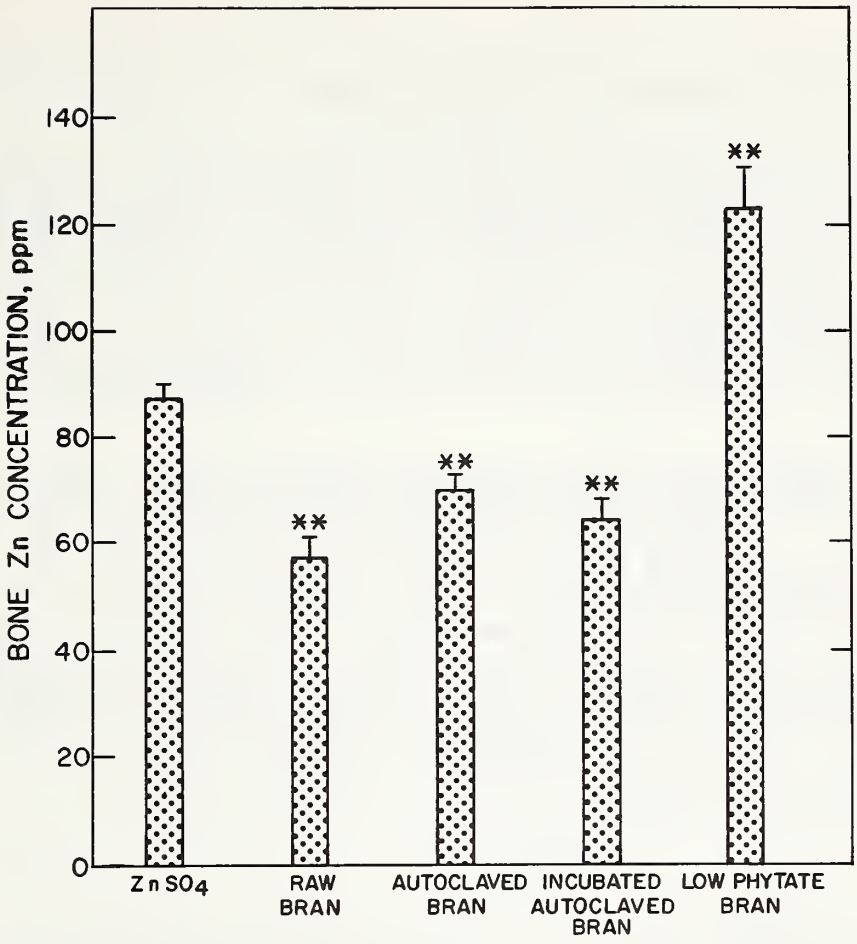

Figure 6. Bone Zinc Response of Rats Fed Wheat Bran Preparations as Dietary Zinc Source. Dietary Zinc concentration was 9 ppm.

**Bone zinc response significantly different from response to $ZnSO_4$, $P<0.01$.

The rats gained less weight when raw bran was the zinc source than when either the low-phytate bran or $ZnSO_4$ was the zinc source (difference statistically significant, $P < 0.01$). The autoclaved brans supported slightly better growth than the raw bran, but the differences were statistically significant only for raw bran vs the autoclaved-incubated bran at 9 ppm of zinc ($P < 0.02$). Low-phytate bran supported better growth than either autoclaved bran. The bone zinc response to the 9 ppm level of dietary zinc is shown in Figure 6. Both $ZnSO_4$ and the low-phytate bran supported higher values of bone zinc than the other three brans. The effect on bone zinc is additional evidence that bioavailability of zinc was the factor that limited growth in this study. The different bran preparations supplied equivalent quantities of dietary fiber to the bran diets.

Discussion and Conclusions

We reduced the concentration of phytate in bran, either enzymatically or by extraction but retained the trace elements iron and zinc. We did not investigate all enzymatic changes that might occur in the bran during the period of endogenous phytase action. Results from the solubility studies and gel filtration chromatography indicate that some proteolytic action probably occurs. Data from the iron bioavailability study supported that observation, and indicated that readily available iron-amino acid complexes may be present in the incubated bran.

The dietary fiber of bran might undergo enzymatic changes. All types of bran however, added similar amounts of dietary fiber to the diets of the rats. In low-phytate bran preparations, the zinc, even in the presence of fiber, was of high biological availability. That finding disagrees with the concept that fiber, per se, decreases bioavailability of zinc (10), but agrees with a recent report by Davies et al (19). The fiber of wheat bran may differ from other fiber types in its effect on mineral absorption (20). In vitro studies indicated that zinc and iron bind to fiber components of wheat bran (21), but our biological studies indicate that low-phytate wheat bran does not seriously diminish the biological availability of zinc to rats.

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Footnotes

[1]Charles River CD ® rats. Trade names are included for the benefit of the reader and do not imply any endorsement of the product by the United States Department of Agriculture.
[2]Unpublished data.

Trace Element Analysis of Wheat Base-Line Samples

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Introduction. In 1974 the Food and Nutrition Board proposed that cereal grains be fortified with nutrients which are potentially deficient among certain population groups. In attempts by industry to implement these proposals, a major question arose that the published data on natural levels of nutrients in cereals were an inadequate base for decisions on levels of fortification of commercial flours of today. Therefore, a base-line study on proposed fortification of cereal grain products was set up by an inter-industry committee under the auspices of the American Baking Association, assisted by the Technical and Nutrition Committee of the Baking Council of Canada. The first task of the committee was the determination of the natural levels of nutrients of concern in wheat, wheat blends, and flour as currently milled.

In the wheat base-line study, samples were collected from various mills in different regions in the United States and Canada according to the sampling procedure described by Ranum and Kulp (1) and by Lorenz and Loewe (2), who determined the mineral compositions of wheat and wheat blend samples. The Nutrient Composition Laboratory in Beltsville received the flour samples for analysis at the end of 1975, a time when methods for the determination of zinc and copper in foods were being set up and evaluated.

Several points regarding the general purpose and protocol of the base-line study need to be examined closely. First, any decisions on the level of fortification must be based upon the analytical data on the natural levels and can only be as valid as both precision and accuracy of that data. This statement is implicitly assumed but not often explicitly stated and considered when decisions on fortification policy are made. Second, as stated in the preliminary report on this study: "No effort was made to minimize the analytical error among laboratories by standardizing assay procedures or by instructing the laboratories to follow a uniform methodology. The objective of this survey is to evaluate the current analytical practice of well established laboratories." (1). This point obviously has a direct bearing upon the first point, the validity of the analytical data. Since a primary mission of the Nutrient Composition Laboratory is to look at the problem of how does one generate and validate accurate, precise data on nutrient composition of foods, we have extensively addressed ourselves to these specific points.

In this article, I will discuss analytical quality control and background work carried out in the Nutrient Composition Laboratory in order to validate food composition data in the area of trace elements, using these wheat base-line study samples as a specific example. Some of the trace element data reported by several other cooperating laboratories in the base-line study will be compared and some preliminary evaluations made of the data as a whole. Example will be made using the zinc content data since this was the most complete available set of data.

Analytical Procedure. Triplicate samples of 0.5 to 1.0 grams were weighed into washed glass test tubes (15 x 150 mm) and 1.0 ml concentrated sulfuric acid (Ultrex, J.T. Baker, Phillipsburg, N.J.) and 1.0 ml distilled water were added. The samples were heated gently (Multitemp Block #2093, Labline Instrument Co., Melrose Park, Illinois) for 1 hour, then 1.0 ml of 50% hydrogen peroxide was carefully added until evolution of bubbles ceased. Additional aliquots of H_2O_2 were added until the solution became clear. The cooled solution was filtered into 15-ml plastic graduated tubes through medium filter paper (Whatman #40) prewashed with dilute HCl. Filter paper containing residue was washed with water, the wash added to the filtrate, and the solution made to 10.0 ml. Aliquots of the filtered solution were diluted by a factor of three or four for analysis by flame atomic absorption spectrometry using a Perkin-Elmer 503 Atomic Absorption Spectrometer equipped with triple slot burner head, air-acetylene flame, under standard operating conditions (zinc, 2319 Å, copper, 3247 Å). An entire laboratory is set up for trace element analysis and particular attention is paid to avoidance of contamination. The analytical procedures are geared for control of contamination at the level of parts per billion. Triplicate samples of standard reference materials SRM 1571 Orchard Leaves and SRM 1577 Bovine Liver from the National Bureau of Standards were run with each set of 10 to 20 unknown samples as a quality control. Results of our analysis of reference materials needed to agree within certified value limits and $\pm$5% RSD for triplicate analyses was required for acceptance of each set of data on the unknown samples.

Discussion. Checking for recovery of added metal and checking for matrix effects by the method of standard additions are common procedures used for validating trace element analytical methods using Atomic Absorption Spectrometry. In our analyses, we recovered 98.1 $\pm$ 1.7% of the copper and 100.5 $\pm$ 2.6% of the zinc added to the sample before digestion. When the results of the method of standard additions of metal to the filtered digest were compared to results from standard calibration curves, the two methods of quantitation agreed within 10%, showing no significant viscosity effects in the atomic absorption analysis of the solutions. These procedures are checks for precision or reproducibility of the method relative to the added metal, but do not "prove" that the intrinsic metal is accurately quantitated.

Conventionally in trace element analyses, once a method has been thus "validated", it is used to analyze a large number of samples on the implicit assumption that as long as the method is precise, it is accurate. Good precision is often the only criterion for control of quality of data generated by a particular method over a period of time. Often there are no control procedures used to insure that the absolute values of the data are accurate on a day-to-day basis. The accuracy of nutrient composition data can only be evaluated by the analysis of primary Standard Reference Materials (SRM) (such as those available from the National Bureau of Standards) using the identical analytical procedure or, conversely, using appropriate standard materials to routinely calibrate the procedure.

Table 1. Zinc and Copper Contents of Standard Materials

	SRM 1577 Bovine Liver		SRM 1571 Orchard Leaves		AACC-1 Wheat	
	Zinc	Copper	Zinc	Copper	Zinc	Copper
	(μg/g)		(μg/g)		(μg/g)	
NCL(N=12)[a]	127.8±8.5	193.3±11.2	28.8±2.8	12.9±1.7	25.9±1.2	4.6±0.6
NBS (certified)	130±10	192±10	25±3	12±1	---	---
AACC-1(N=8)[a]	---	---	---	---	25.3±3.1	4.7±0.9

NCL = Nutrient Composition Laboratory, NBS = National Bureau of Standards
AACC = American Association of Cereal Chemists. (a) = Values are mean ± SD

Data generated on the SRM check samples for initial and day-to-day validation of assay procedures for zinc and copper are presented in Table I. Some standard wheat samples prepared for and used in a collaborative study of the American Association of Cereal Chemists (AACC) method 40-70 (Elements by Atomic Absorption Spectrometry) were utilized as further check samples of a biological matrix more closely representing the wheat flour base-line samples. Values of the mean and standard deviation of data from 8 different laboratories were used as check values for these samples.

Both the SRM and standard wheat check samples were run with each of six batches of unkown samples necessary to complete the analysis of the base-line study samples. The wheat check samples and, with one exception, the SRM check samples all fell within the uncertainty ranges for those samples. Thus quality control of the accuracy of the analytical data was validated for each separate set of unknown samples.

Table 2. Zinc and Copper Content of Wheat Flour Base-Line Study Samples

Sample Number	Zinc (μg/g)	Copper (μg/g)	Sample Number	Zinc (μg/g)	Copper (μg/g)
F-10	7.4 ± 0.2	1.23 ± 0.08	F-54	8.9 ± 0.0	2.12 ± 0.11
F-11	7.4 ± 0.1	3.49 ± 0.27	F-55	7.9 ± 0.5	1.71 ± 0.06
F-12	9.4 ± 0.2	2.91 ± 0.20	F-56	4.7 ± 0.4	1.29 ± 0.18
F-13	9.2 ± 0.0	5.27 ± 0.33	F-57	12.3 ± 0.7	2.52 ± 0.17
F-14	10.1 ± 0.1	2.74 ± 0.23	F-58	10.2 ± 0.4	2.40 ± 0.29
F-15	8.4 ± 0.2	1.96 ± 0.04	F-59	6.7 ± 0.3	1.63 ± 0.14
F-16	7.0 ± 0.2	1.33 ± 0.30	F-60	5.2 ± 0.4	1.44 ± 0.06
F-17	7.4 ± 0.0	1.08 ± 0.11	F-61	7.1 ± 0.4	1.27 ± 0.10
F-21	6.5 ± 0.2	2.33 ± 0.07	70	10.3 ± 0.2	2.02 ± 0.14
F-22	8.8 ± 0.2	1.19 ± 0.12	71	5.9 ± 0.3	1.50 ± 0.03
F-23	9.3 ± 0.4	2.26 ± 0.07	72	7.6 ± 0.3	1.38 ± 0.02
F-25	9.9 ± 0.6	2.01 ± 0.11	73	9.3 ± 0.3	1.82 ± 0.03
F-26	7.4 ± 0.2	2.19 ± 0.11	74	7.4 ± 0.6	1.67 ± 0.04
F-27	7.8 ± 0.3	1.32 ± 0.22	75	7.4 ± 0.2	1.85 ± 0.04
F-28	7.5 ± 0.3	1.20 ± 0.08	76	4.8 ± 0.0	1.38 ± 0.35
F-29	6.5 ± 0.1	2.26 ± 0.33	77	7.4 ± 0.3	1.78 ± 0.14
F-30	4.0 ± 0.2	0.86 ± 0.14	78	11.4 ± 0.2	2.02 ± 0.17
F-34	5.8 ± 0.4	2.28 ± 0.22	79	9.4 ± 0.6	1.76 ± 0.13
F-37	9.3 ± 0.6	2.62 ± 0.14	81	7.7 ± 0.5	2.00 ± 0.08
F-40	8.0 ± 0.5	1.76 ± 0.21	82	7.4 ± 0.5	1.68 ± 0.06
F-41	5.3 ± 0.1	1.08 ± 0.07	83	6.0 ± 0.1	1.34 ± 0.04
F-43	6.6 ± 0.4	1.46 ± 0.09	84	6.5 ± 0.0	1.51 ± 0.11
F-44	7.9 ± 0.2	2.17 ± 0.19	85	9.8 ± 0.6	1.86 ± 0.07
F-45	7.0 ± 0.5	1.30 ± 0.11	86	6.3 ± 0.3	1.66 ± 0.23
F-46	12.5 ± 0.3	1.76 ± 0.08	87	7.9 ± 0.1	1.91 ± 0.11
F-47	8.2 ± 0.5	1.79 ± 0.14	01	4.7 ± 0.3	1.11 ± 0.13
F-48	9.4 ± 0.3	1.99 ± 0.25	02	8.4 ± 0.5	2.28 ± 0.34
F-49	8.5 ± 0.1	2.10 ± 0.05	04	11.8 ± 0.1	2.55 ± 0.16
F-50	14.4 ± 0.6	2.23 ± 0.06	13	12.5 ± 0.8	1.46 ± 0.18
F-51	3.9 ± 0.2	1.09 ± 0.18	L4X	7.2 ± 0.30	2.06 ± 0.08
F-52	8.6 ± 0.2	1.39 ± 0.10	L5X	4.6 ± 0.1	0.83 ± 0.13
F-53	8.5 ± 0.4	2.79 ± 0.28	B1	8.0 ± 0.1	2.59 ± 0.34

Values are mean ± SD of three replicate analyses.

In Table 2 are listed the analytical results for the individual base-line study flour samples.

In a collaborative, round robin, or cooperative study, samples are collected and sent to the collaborators, who analyze the samples, and the results are returned to the primary investigators, who compile the data. Invariably, the first time that samples are distributed, the results from different laboratories disagree and replicate samples are redistributed, until agreement is reached on the "correct" values for the samples.

If the proper quality control is utilized and the results are referenced to appropriate bench mark or primary standard samples, then the evaluation and comparison of data accuracy from individual laboratories is possible. Since the data in Table 2 fulfill these criteria they will be used as an accuracy reference point in evaluating data accumulated by other laboratories on the base-line samples. These reference data are designated as from Laboratory J in the following discussions.

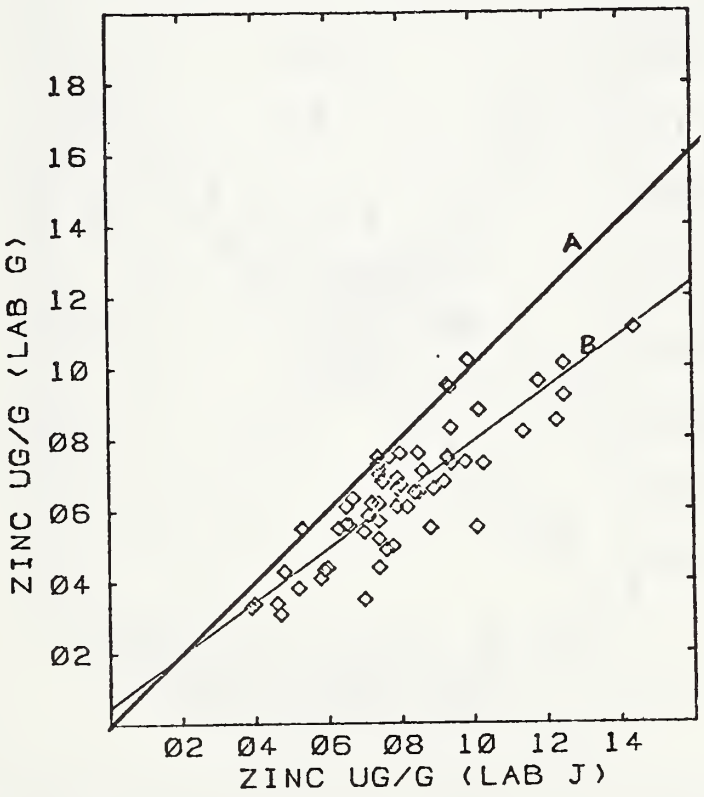

Figure I. Comparison of zinc levels of wheat flour base-line samples. Line A (Y = X), Line B (Y = 0.746X +0.48 μg/g).

Figure I shows the data from Laboratory J plotted on a one-to-one basis with the zinc data from another laboratory (G). If the data were in perfect agreement on every sample all the points would fall on line A (Y = X) with a slope of 1.0 and an intercept of zero. As the figure actually shows, the results all fall below line A. This means that the results from Laboratory G are low relative to those of Laboratory J. The high correlation coefficient of 0.859 of the linear regression line B (Y = 0.746X +0.48) for this large number of points (n = 65) means that there is a very good one-to-one correlation between the two sets of data. The low intercept of line B (0.48 µg/g) is probably within experimental error. The slope of 0.746 indicates approximately a 25% relative difference between the two laboratories. Thus these two sets of data are well correlated with a possible 25% relative bias and a very small absolute bias.

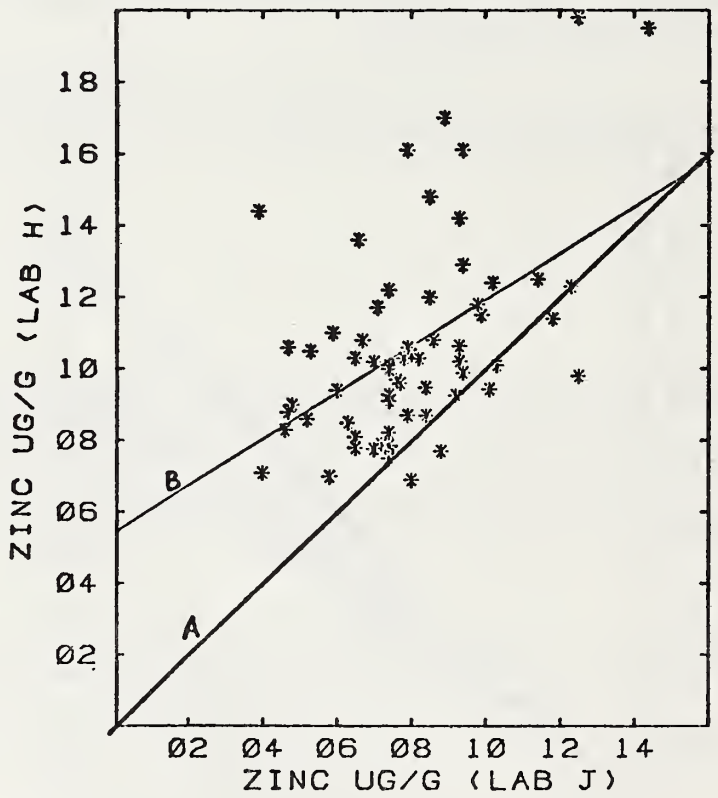

Figure II. Comparison of zinc levels of wheat flour base-line samples. Line A (Y = X), Line B (Y = 0.648X +5.45 µg/g).

Figure II shows a similar set of data, comparing again Laboratory J with data from Laboratory H, in which it can be seen that all of the points fall above line A (Y = X), meaning that there is almost no overlap of the results from Laboratory G and H. Also the much lower correlation coefficient (0.495) of the linear regression line B (Y = 0.648X +5.45µg/g) indicates a much poorer one-to-one agreement with the data from Laboratory J. The slope is approximately 35% low with a very high intercept. The high intercept, large scatter, and lower slope all indicate that the analytical procedure is not as well under control as in the comparative Laboratory J. The existence of a large positive absolute bias might arise from an unrecognized or uncorrected problem with analytical blanks.

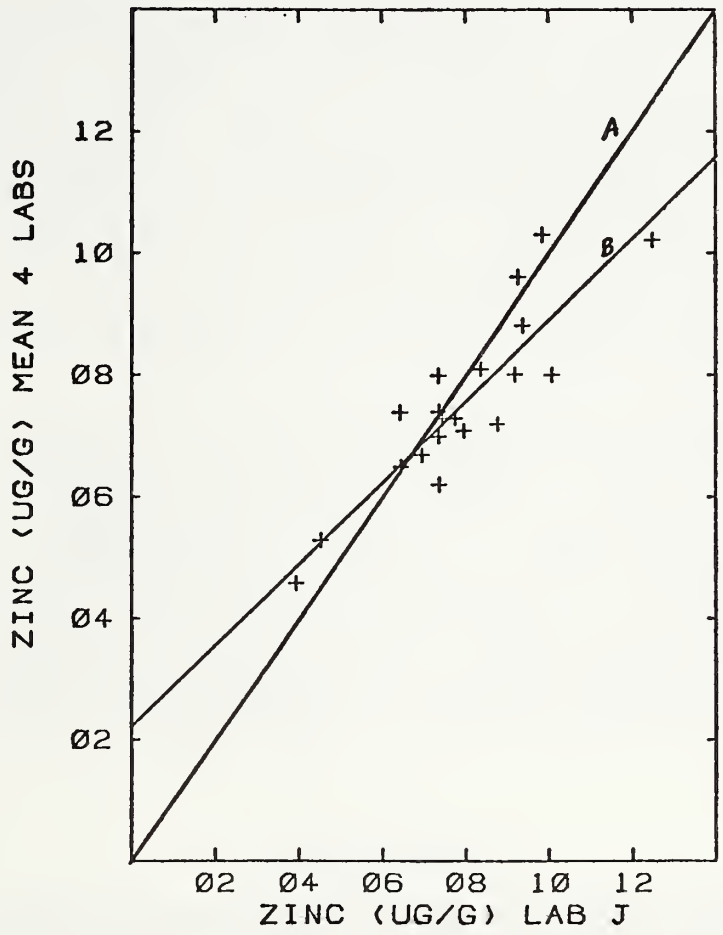

Figure III. Comparison of zinc levels - Mean values vs. Reference Laboratory Values. Line A (X = Y), Line B (Y = 0.67X +2.2µg/g).

This type of evaluation can be of help in comparing the data from individual laboratories, however, the reports of a collaborative study often discuss the mean or average values, not the individual values, of the contributing laboratories. In Figure III the mean values from four laboratories are plotted against the individual reference values of Laboratory J. The correlation ($r = 0.886$) is good, and the linear regression line B ($Y = 0.67X + 2.2$ µg/g) has a somewhat low slope and a significant intercept. Thus even the combined data can have an absolute uncertainty of 2.2 µg/g and relative error of 33% compared to the reference data from Laboratory J, especially at the high and low values.

If the accuracy of the mean data is taken as valid within the uncertainty expressed in the certified range for the SRM's represented by the data from Laboratory J, then some evaluation can be made of the consequences of uncertainty in the mean values expressed in Figure III. For example, the decisions on fortification of bread flour with zinc are expressed as a proposed level of 10 mg zinc per pound. For bread with a natural level of 3.5 ± 0.5 mg/lb, based on the error observed in precision between laboratories, the deficit is 7.0 mg/lb and a proposed fortification level of 7.0 mg/lb is recommended (3). If the natural levels as expressed in the base-line samples are in positive absolute error of 2.2 µg/g or 1.0 mg/lb and/or a relative error of 33%, then the uncertainty in the natural level of the above example become 3.5 ± 1.0 mg/lb, the deficit becomes 7.5 mg/lb and the addition level becomes about 0.5 mg/lb larger than indicated above.

Summary. The criterion of proper quality control involving use of standards referenced to primary standard materials is necessary for setting up a valid base-line study to be used in making decisions on fortification of foods or other policy with far-reaching effect or impact. These standards are becoming available in the area of trace element content of foods and a new Standard Reference Material (1567 Wheat Flour) has been recently released by the National Bureau of Standards (4). This material should be used and promoted by national user organizations in studies such as the wheat base-line study so that the analytical data can be properly validated and uncertainty expressed on basis of absolute error in addition to relative error. Thus, fortification policy decisions can be based on sound scientific discussion.

Acknowledgment

The assistance of Frank Hepburn in initiating this study and in discussions regarding its implications, and the assistance of Doris Baker in supplying the AACC collaborative wheat samples and analytical data is gratefully acknowledged.

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ALKYLRESORCINOLS IN CEREAL GRAINS

by

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"Is rye poisonous?" is the title of a 1974 article in Getreide, Mehl und Brot - a cereal chemistry journal published in Germany. Birds do not touch kernels of rye. Pigs and chickens can tolerate only a certain percentage of rye in feed rations, the article states. Is there a possibility that the consumption of rye bread can be detrimental to health, caused by derivatives of resorcinol isolated from rye in 1967?

Rye bread consumption in Germany has decreased over the last few decades, and, therefore, the intake of alkylresorcinols from rye, while life expectancy has increased. Is there a connection?

Papers such as the one cited above have caused the recent interest in alkylresorcinols.

The Growth-Inhibiting Effect of Rye.

It is well known that rye, when fed in large amounts to cattle, sheep, horses, pigs and poultry, leads to slower growth in comparison with feeding of other cereal grains (1). Of the symptoms caused by rye feeding, the feed intake inhibiting effect is mentioned in all articles on the effects of rye feeding on pigs and poultry (2, 3).

The growth inhibiting effect of rye has often been ascribed to ergot (Claviceps purpurea) contamination. Rations with toxic levels of ergot exceeding 1% are so unpalatable, however, that most animals refuse to eat them (4). The effect of ergot, therefore, must not be mistaken for the effect of rye itself.

Results of Honcamp (3) with pigs indicated that the average daily feed intake of a diet containing 67% rye, 23% barley and 10% fishmeal was lower than the intake on 90% barley and 10% fishmeal. The daily gain of the rye fed animals was 12% lower, whereas the feed conversion was 9% higher than that of the controls. Wilkens (5) found that the growth of pigs on rations containing up to 50% rye was 8% lower than the growth of barley - or corn fed animals. In general, young animals are more susceptible to the growth inhibiting effect of rye than older ones. Rye is also less palatable to chicks than wheat, corn or barley.

In each of the studies it was realized that the diminished growth rate was caused primarily by a decrease of feed intake, which some researchers blamed on

the bitter taste of rye, but the question remained whether the lowering of the feed intake was entirely caused by this bitter or disagreeable taste or whether one as yet unknown physiological effect on the animal body was involved. In 1957 van Wieringen (6) conducted paired feeding experiments with rats on diets of 90% rye or barley and 10% casein. Growth was equal in both groups when the feed intake of the animals on the barley diet was restricted to a level corresponding to the feed intake of the rye fed animals. There were no differences in digestibility. After 3 weeks on the respective diets, all animals received the barley ration ad libitum. It was observed that 6 out of 9 animals, originally on the rye diet, did not eat as much of the barley diet as did the rats which always received the barley diet. From the results of this study it was concluded that the reduced feed intake is not due to the bitter taste of rye but due to an unknown component in rye which affects the constitution of the animal.

The Toxic Factor

In 1967 Wieringa (7) identified the harmful substances in rye as a mixture of 5-n-alkylresorcinols with odd numbered side chains of 15-23 carbon atoms and of smaller amounts of 5-alkenylresorcinols. These compounds were primarily concentrated in the pericarp of rye kernels. Fig. 1 shows the chemical structure of the toxic substance in rye. They were identified by gas-liquid chromatography and mass spectrometric analyses.

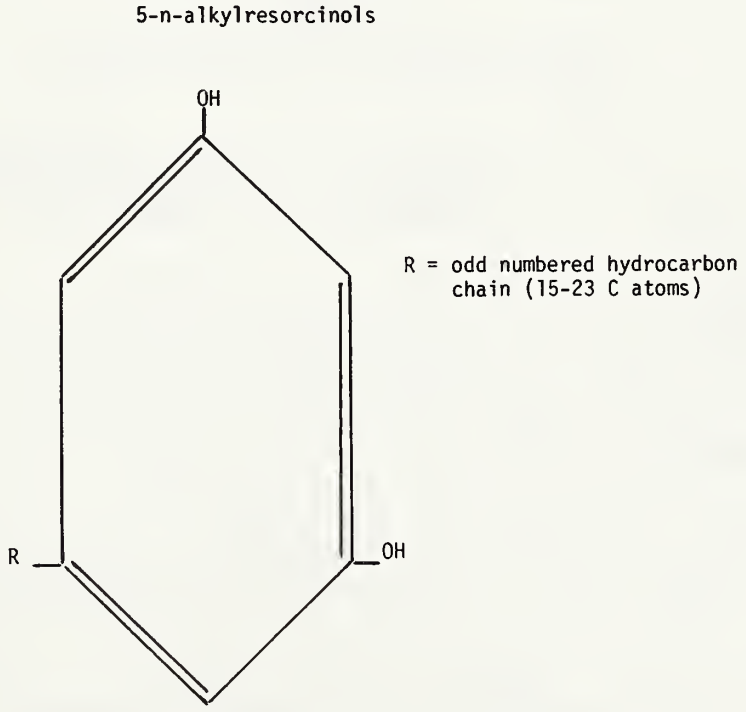

Fig. 1. Chemical structure of 5-n-alkylresorcinols.

In 1964 Wenckert et al. (8) had found 5-n-alkylresorcinols in wheat bran. The NMR spectrum pointed to resorcinol derivatives with a side chain of 17, 19, 21, 23, and 25 carbon atoms.

Alkyl derivatives of dihydroxybenzene are not uncommon in nature. They are found also in species of the Anacardiaceae, to which, for instance, poison ivy or cashew nuts belong (9). Species belonging to this family are known to contain catechol or resorcinol derivates in the sap, the leaves or the fruit (7).

It is obvious from the work of Wenckert et al. (8) and that of Wieringa (7) that both wheat and rye contain 5-alkylresorcinols. No difference in growth inhibiting effect could be detected between rye and wheat resorcinols (7). Why then has rye a bad reputation as a feedstuff while wheat has not? The answer very likely seems to be concentration of the toxic component. Wheat bran resorcinols, however, may still play a part in animal feeding, but the effects are not quite as obvious because of lower concentrations, as indicated by the recommendation that wheat, although an excellent feed for pigs, should not be used at amounts higher than 50% of the ration (7).

Alkylresorcinols in Grains

The alkylresorcinol index of several varieties of wheat, triticale and barley of eastern European origin was reported by Stuczynski et al. (10) and is shown in Table 1.

TABLE 1

ALKYLRESORCINOLS IN GRAIN OF CEREALS

Cereal	Number of Samples	Index of Alkylresorcinol Content	
		Range	Average
Rye	8	326-441	370
Wheat	5	134-194	177
Triticale	16	192-288	229
Barley	2	44- 52	48

Source: Stuczynski, E., Jakubowski, S. and Stuczynaska, J. Hodowla Roslin, Aklimatyzacja I Nasiennictwo 18(4), 287 (1974).

Wheat contained approximately 48%, triticale 62% and barley 13% of the alkylresorcinols in rye.

The alkylresorcinol contents of some grains from the U.S. and Canada, as determined by Evans et al. (11) are shown in Table 2. Of the wheats, Selkirk had the highest and Stewart 63 the lowest amount. The range in wheat was less

TABLE 2

5-ALKYLRESORCINOL CONTENT IN THE SEED
OF SOME SELECTED GRAIN SPECIES

Species	Cultivar	Alkylresorcinol content (% pentadecylresorcinol)
Rye	Prolific	.097
Triticale	Rosner	.079
Wheat	Stewart 63	.053
	Pitic 62	.079
	Manitou	.060
	Kenya Farmer	.055
	Neepawa	.064
	Selkirk	.089
Barley	Conquest	.010
Millet		.010

Source: Evans, L. E., Dedio, W., and Hill, R. D. Can. J. Plant Sci. 53, 485 (1973).

than found in rye and triticale. Barley and millet had negligible levels of these compounds. The data by Stuczynski et al. (10) and Evans et al. (11) show differences in alkylresorcinol content between varieties. The genetic nature of these differences has not been proven. There have been indications that factors such as soil type, fertilization and weather influence the amounts of alkylresorcinols (7). The data also point out that some wheat varieties approach alkylresorcinol contents of rye varieties. The wheat variety Selkirk in the study of Evans et al. (11) contained only slightly lower amounts of the compounds than Prolific rye.

The finding that the resorcinol derivatives are located primarily in the pericarp is important. The amounts of these compounds in cereals might, therefore, be proportional to the surface area of the kernel and not to the weight (7). It is known that due to agronomic and environmental conditions, a variation in kernel size and weight occurs, which might make it difficult to interpret differences in 5-n-alkylresorcinols of cereal grain varieties.

Cereal grains grown at the same location during consecutive crop years show only slight variations in alkylresorcinol from year to year as seen in Table 3. The alkylresorcinol values reported by Verdeal and Lorenz (12) were

TABLE 3

EFFECT OF CROP YEAR ON
ALKYLRESORCINOL CONTENT (% DRY WEIGHT)
OF CEREAL GRAINS

Grain Sample	Crop Year 1972	1973	1974	1975
HRS Chris Wheat	0.062	0.065	--	--
HRS Colano Wheat	--	--	0.067	0.067
Triticale 6-TA-204	0.085	0.082	0.095	0.095
Triticale 6-TA-206	0.070	0.079	0.077	0.075
Prolific Rye	0.124	0.105	0.101	0.108

From: Verdeal, K. and Lorenz, K. Cereal Chem. 54, 475 (1977).

in the same range as those of Evans et al. (11). Statistically significant differences due to crop year were found only in a few instances.

Alkylresorcinols in Milling Fractions

Thus far, alkylresorcinols have only been mentioned as being detrimental factors in animal nutrition, although concern has been occasionally raised about possible ill-effects on humans (especially young children) in countries of Europe with high rye bread consumption (13). There are not established toxicity levels for humans for these compounds.

The consumption of whole grain cereal products is relatively low in most developed countries. Cereal grains are dehulled, milled, polished or processed in many ways, depending on the grain, and separated into fractions, some of which are used as only animal feed. This processing results in a shift of the alkylresorcinols, originally present in the grain, into specific milling fractions. Disecting kernels of grain, Wieringa (7) found that alkylresorcinols are found primarily in the pericarp of grains. This part of the kernel would become part of the bran fraction. That this is so was shown by Verdeal and Lorenz (12), who analyzed milling fractions of wheat, rye, and triticale obtained by milling these grains on a Quadrumat Sr. mill. Their data are shown in Table 4.

The bran fractions contained the highest, shorts intermediate and the flour fractions the lowest amounts of alkylresorcinols, which would indicate that a gradient exists with the highest amounts of the compounds in the pericarp, intermediate amounts in the aleurone layer and relatively small but detectable amounts in the endosperm portion of cereal grain kernels.

TABLE 4

ALKYLRESORCINOL IN MILLING FRACTIONS (% DRY WEIGHT)
(QUADRUMAT SR. MILL)

Grain Sample	Milling Fraction - (%)		Alkylresorcinol (%)
Wheat - Chris	Bran	19.6	0.211
	Shorts	6.9	0.070
	Flour	73.5	0.038
Triticale 6-TA-204	Bran	27.7	0.213
	Shorts	6.0	0.087
	Flour	64.8	0.026
Rye-Prolific	Bran	33.6	0.186
	Shorts	10.5	0.115
	Flour	52.0	0.031

From: Verdeal, K. and Lorenz, K. Cereal Chem. 54, 475 (1977).

In the past, wheat bran, which contains the highest amounts of alkylresorcinols, was used as animal feed. Today, however, with the emphasis on higher fiber intake, this wheat bran may be used in various food products to raise the fiber level, since cellulose and its derivatives, which are the only well defined products containing a single type of fiber, have not been acceptable to certain consumer groups. It is realized that in most instances the wheat bran will be used in small quantities, but still it means that a product is used in our foods which contains more than twice the level of alkylresorcinols in rye and we know that the level of alkylresorcinols in rye is a detrimental factor in animal nutrition.

What is the level of alkylresorcinols in all-bran breakfast cereals? These resorcinol derivatives are fairly heat stable. Are they destroyed during heat processing? We do not have the answer to these questions at this time.

Alkylresorcinol in Breads

The alkylresorcinol contents of crust, crumb, and whole loaves baked from whole grain meals of Prolific rye, triticale 6-TA-204 and Chris wheat are shown in Fig. 2.

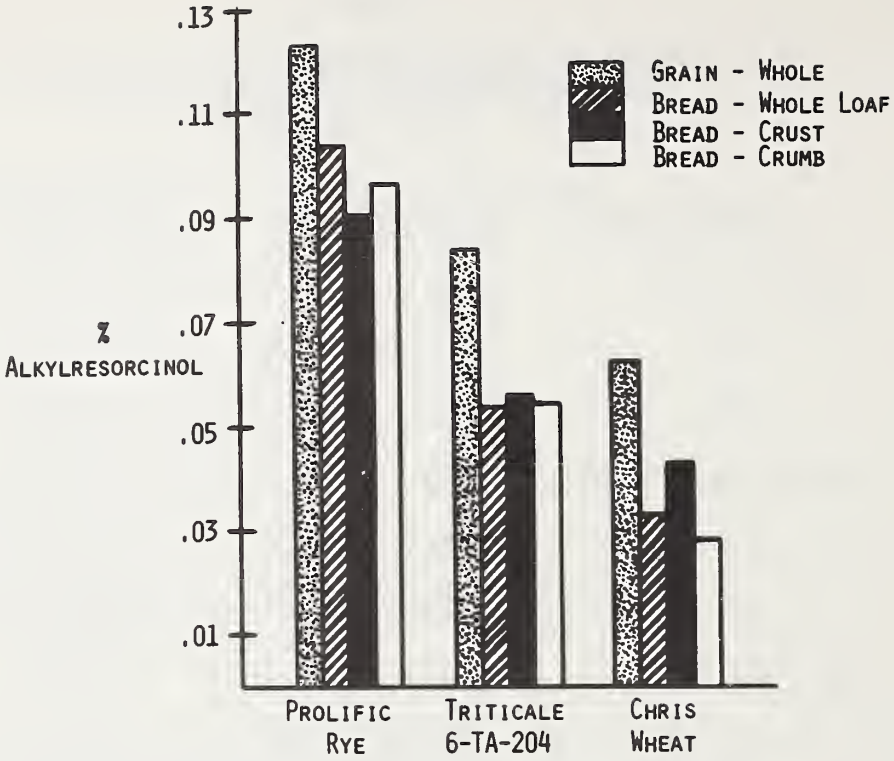

Fig. 2. Alkylresorcinol content of whole grain meals and breads.

Due to the uneven distribution of alkylresorcinols in grain kernels, which leaves only relatively small amounts of the compounds in the flour fraction after milling, whole grain meals were used in baking experiments to determine the effects of temperature, reached during baking, on alkylresorcinol retention (12). It was found that the amounts of alkylresorcinols were lower in the breads and in the crusts and crumb portions of the breads than in the whole grain meal before baking. There was a uniform loss of alkylresorcinols throughout the loaves, as shown for the triticale 6-TA-204 breads. If temperature were a factor in the loss of these compounds, the crust of a loaf, which is exposed to a higher temperature and for a longer period of time at that temperature, than the crumb during the baking of bread, should consistently contain lower amounts of alkylresorcinols, which it did not. The small differences in alkylresorcinol content between crust, crumb and the entire loaf of the Prolific rye and Chris wheat breads are believed to be due to sampling. Losses in percent alkylresorcinol are shown in Table 5.

It was concluded that the loss of alkylresorcinols was independent of the temperature reached during baking and probably occurred as the result of fermentation (12).

Alkylresorcinol loss during the entire baking process was an absolute amount unrelated to the initial amount present in the whole grain meal, which led to the speculation that the loss occurs during fermentation. Absolute

TABLE 5

ALKYLRESORCINOL LOSSES DURING BAKING

	Alkylresorcinol (%) in Whole Grain	in Bread	Alkylresorcinol loss (%) during Baking
Prolific Rye	0.124	0.096	0.028
Triticale 6-TA-204	0.085	0.055	0.030
Chris Wheat	0.062	0.036	0.026

losses, in the study by Verdeal and Lorenz (12) were 22% for rye, 24% for triticale 6-TA-204 and 23.5% for Christ wheat.

Conculsions

The alkylresorcinols in cereal grains have become of interest only recently. It was believed that these compounds are mainly associated with rye and that they present a problem only in animal nutritionl Since cereal grains, including rye, are milled into flour before being used in food applications, there was little concern. Alkylresorcinols are concentrated in the pericarp, which becomes part of the bran fraction. This fraction is used for animal feed. Recent analyses for alkylresorcinols in several U.S. and Canadian wheats, however, have shown that levels of these compounds approaching those in rye are found in certain varieties. Milling of these wheats will produce bran fractions with rather high alkylresorcinol contents, which might be used in high fiber products because of the alleged benefits of higher levels of this nutrient in the diet of man. Thus, alkylresorcinol intake through certain high fiber foods could increase. Since it is known that alkylresorcinols have detrimental effects on animal metabolism, their effects in human nutrition deserve further attention.

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WHEAT GLUTEN - ALKALI REACTIONS

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Abstract

Treating commercial wheat gluten under alkaline conditions at 65°C for various time periods destroys part of the serine, threonine, cystine, lysine, arginine, and tyrosine residues. The lossess were accompanied by the appearance of lysinoalanine and unidentified ninhydrin-positive substances. Amino acid analysis of alkali-treated-acylated wheat gluten revealed that acylation by acetic and succinic anhydrides prevents or minimizes destruction of lysine residues and the formation of lysinoalanine. Mechanistic hypotheses are offered for the observed formation and nonformation of lysinoalanine.

Introduction

Alkali-treatment of food proteins has been used for many purposes including preparing meat analogue vegetable (soy) protein (1, 2), destroying aflatoxin (3), peeling fruits and vegetables (4, 5), and preparing protein concentrates (6, 7).

Crosslinked amino acids have been found in hydrolysates of both alkali-treated and heat-treated proteins (2, 8, 9). One of these crosslinked amino acids, lysinoalanine, has been found to cause histological changes in the descending portion (pars recta) of the proximal tubules of rat kidneys (10-12). These observations have caused concern about the nutritional quality and safety of alkali-treated food proteins. There is a need to explain the chemical changes that produce "unnatural" amino acids such as lysinoalanine and lanthionine during alkali treatment, and there is a need to develop strategies to minimize or prevent these reactions.

In previous papers we have (a) analyzed factors that are expected to operate during alkali-induced crosslinking of amino acid residues in proteins (8); (b) demonstrated inhibitory effects of sulfite ions and mercaptoamino acids such as cysteine on lysinoalanine formation during alkali treatment of casein, lysozyme, soy protein, trypsin inhibitor, and wool (8, 13); (c) observed alkali-induced changes in the amino acid composition of soy protein (14); (d) studied the reaction of native and reduced proteins and of polyamino acids with dehydroalanine (8, 15); and (e) critically reviewed effects of lysine modification on chemical, physical, nutritional, and functional properites of proteins and related amino acids and peptides (16). In this paper, I discuss the effect of alkali on complete amino acid profiles of commercial wheat gluten and its acetylated and succinylated derivatives. The results show that acylation protects proteins against lysinoalanine formation.

Materials and Methods

Commercial wheat gluten was obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.

Acetylated Wheat Gluten. Gluten (12g) suspended in 100cc of saturated sodium acetate solution to which 100cc of water had been added, was placed in an ice bath. The suspension was maintained at 0°C. Acetic anhydride (20cc) was added with stirring from a dropping funnel over a period of about ninety minutes (about 40 drops every ten minutes). The suspension was stirred for another two hours, dialyzed for two days against distilled water, and lyophilized.

Succinylated Wheat Gluten. Gluten (10g) suspended in 300cc of water was cooled to 0°C in a water-sodium chloride ice bath. A solution of 0.01 N NaOH was added to the gluten suspension until the pH reached 7.0. Succinic anhydride (7g) was added in seven portions over a period of 70 minutes. The pH was maintained at 7 by addition of 1 N NaOH. The reaction mixture was stirred for another hour, dialyzed against 0.01 N acetic acid, and lyophilized.

Examination of acetylated and succinylated wheat gluten and of native gluten by the mannual ninhydrin reaction (19-21) showed that the amino groups had been modified to the extent of about 80-85%.

Alkali Treatments. Borate buffers of appropiate pH were prepared by adding 0.1 N NaOH to a 0.05 M sodium borate (borax) solution. A solution or suspension of gluten or gluten derivative (for a 1% solution, 0.5 gram of protein per 50cc of solvent; for a 10% solution, 5.0 grams per 50cc) in a glass-stoppered Erlenmeyer flask was placed in a 65°C water bath. After the indicated time, the solution was dialyzed against 0.01 N acetic acid and lyophilized.

Amino Acid Analyses. A weighed sample of gluten (about 5 mg) was dissolved in 15cc of 6 N HCl in a commercial hydrolysis tube. The tube was evacuated, placed in an acetone-dry ice bath, evacuated and refilled with oxygen-free nitrogen twice before being placed in an oven at 110°C for 24 hrs. The cooled hydrolysate was filtered through a sintered disc funnel, evaporated to dryness at 40°C with the aid of an aspirator, and the residue was twice resuspended in water and evaporated to dryness. Amino acid analysis of an aliquot of the residue was carried out on a Durrum Amino Acid Analyzer, Model D-500, under the following conditions: single-column Moore and Stein ion-exchange chromatography. Resin, Durrum DC-4A; buffer pH, 3.25, 4.25, 7.90; photometer, 440 nm, 590 nm; column: 1.75 mm X 48 cm; analysis time: 105 minutes. Norleucine was used as an added internal standard.

In this system, lysinoalanine elutes just before histidine. The color constant of lysinoalanine was determined with an authentic sample purchased from Miles Laboratories, Elkhart, Indiana. The results are summarized in Figures 1 and 2 and Tables 1-5.

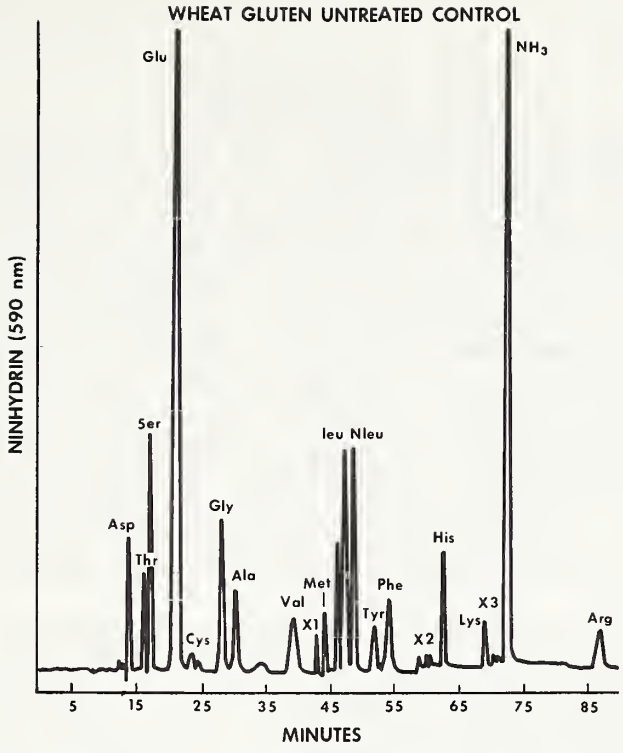

Figure 1. Amino acid analysis of a hydrolysate of commercial wheat gluten.

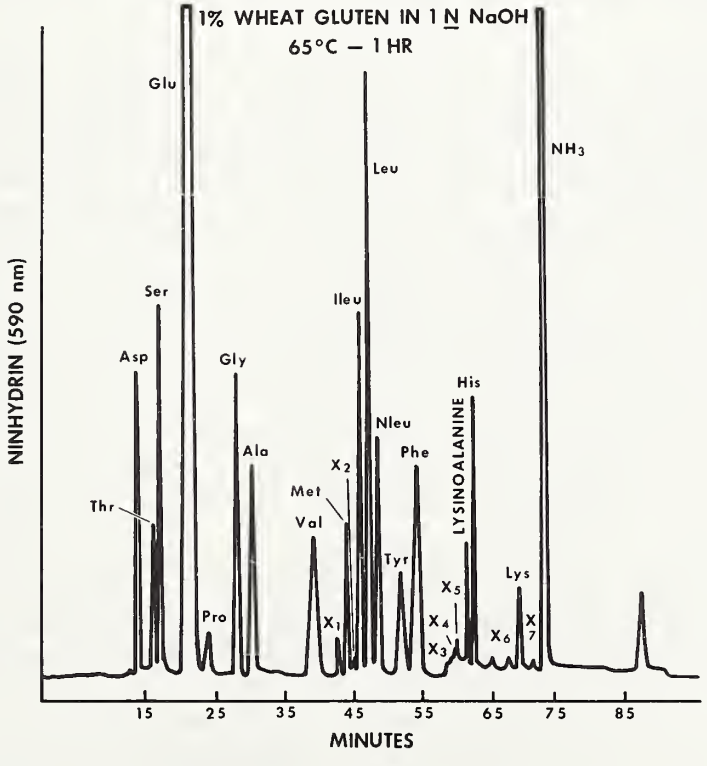

Figure 2. Amino acid analysis of a hydrolysate of alkali-treated commercial wheat gluten.

Results and Discussion

Effects of pH and Gluten Concentration. The results in Tables 1-5 are reported in terms of mole (residue) per cent, defined as moles of each amino acid recovered from the ion-exchange column divided by the sum for all amino acids and in terms of mole ratios to alanine (alanine or any other amino acid from the same analysis that is unaffected by the treatment acts as an internal standard) rather than in terms of weight of each amino acid per weight of protein. The mole per cent and ratio methods are better measures of amino acid composition of commercial, processed, or chemically modified proteins because they avoid errors due to moisture content (it was noted that the acylated gluten derivatives are highly hygroscopic), protein content (the commercially used wheat gluten contains carbohydrates, lipids, etc. which may be lost during the work-up of the reaction mixture), molecular weights (partial hydrolysis undoubtedly takes place during the alkaline treatment of the wheat gluten), and errors occurring in all operations during amino acid analysis (Cf., 22).

The data show that the following amino acids are degraded or destroyed during the alkaline treatment of gluten: threonine, serine, cystine, tyrosine, lysine, and arginine. Thus, treating 1%(w/v) gluten in 1 N NaOH (Tables 1, 2) at 65°C decreases threonine to about 70% of the original value after one hour, 39% after three hours, and 25% after eight hours. The corresponding values for serine are 63, 36, and 24%; for tyrosine, 84, 76, and 68%; for lysine, 67, 76, and 87%, and for arginine, 82, 69, and 42%, respectively. The treatment also induces the disappearance of cystine and the appearance of lysinoalanine (LAL) residues.

The decrease in lysine appears to go through a minimum. This unexpected result may be due to the fact that the lysine peak is a composite of lysine and ornithine, the latter being derived from alkali-degradation of arginine (8). A more likely possibility is that lysinoalanine is destroyed during the alkaline treatment, regenerating lysine. (This has previously been shown to be the case when analogous cyanoethyl derivatives of lysine are subjected to alkaline conditions) (23). The data suggest that after about one hour, the rate of lysinoalanine destruction may be as fast as its rate of formation. A dynamic equilibrium is thus established between lysine and lysinoalanine. This conclusion is supported by the similar leveling off of lysinoalanine content after about one hour in promine-D (soy protein) and lactalbumin. In addition, it was noted that destruction of alkali-labile amino acids appears to proceed at similar, although not identical, rates compared to gluten when these two proteins are subjected to analogous alkaline conditions.

The amino acid composition and conformation of a specific protein, as well as the size of the protein chains, all may influence its reactivity with alkali. It is, therefore, not surprising to find differences in rates of destruction of alkali-labile amino acids in different proteins. What is surprising is that the concentration of the substrate (e.g., wheat gluten) does not appear to affect the extent of alkali-induced change in amino acid composition (of the treated protein) or the rate of formation of lysinoalanine. Thus, data in Tables 2 and 3 show that alkali-induced changes in amino acid composition

Table 1. Effect of pH on amino acid composition of wheat gluten.

Conditions: 1% wheat gluten; 65°C; 3 hours.

Numbers are mole (residue) per cent[a] for each amino acid.

	Control	pH 9.6	pH 10.6	pH 11.2	pH 12.5[b]	pH 13.9[c]
ASP	3.20	3.26	3.26	3.15	3.57	2.96
THR	3.19	3.10	3.05	3.01	2.67	1.20
SER	6.81	6.75	6.64	6.55	5.30	2.24
GLU	27.90	32.57	32.53	33.36	33.09	39.28
PRO	18.33	14.25	14.80	14.82	13.56	16.55
GLY	6.01	6.30	5.78	6.10	5.76	3.94
ALA	3.91	3.95	4.07	3.86	4.40	3.78
CYS	0.976	0.691	0.00	0.00	0.00	0.00
VAL	4.36	4.32	4.40	4.26	4.80	4.20
MET	1.35	1.25	1.18	1.33	1.68	1.17
ILEU	3.68	3.52	3.68	3.62	4.37	3.97
LEU	7.48	7.28	7.49	7.31	8.00	8.58
TYR	2.49	2.55	2.43	2.49	2.43	1.85
PHE	4.35	4.29	4.52	4.35	4.29	4.93
LAL	0.00	0.00	0.262	0.420	0.762	0.884
HIS	1.87	1.80	1.83	1.78	1.74	1.71
LYS	1.33	1.40	1.16	0.963	0.945	0.948
ARG	2.75	2.70	2.66	2.68	2.61	1.79

[a]Mole per cent is defined as moles of each amino acid recovered from the ion-exchange column divided by the sum for all amino acids listed times 100.

[b]pH of Protein in 0.1 N NaOH

[c]pH of protein in 1.0 N NaOH

Table 2. Effect of alkali treatment on amino acid composition of gluten.

Conditions: 1% wheat gluten in 1N NaOH; 65°C.

A columns are mole per cent and B columns mole ratios to alanine.

Amino Acid	Gluten Control (untreated)		Time of Treatment					
			1 HR		3 HR		8 HR	
	A	B	A	B	A	B	A	B
ASP	3.20	0.819	3.28	0.801	2.96	0.810	2.77	0.825
THR	3.19	0.816	2.34	0.573	1.18	0.322	0.685	0.205
SER	6.81	1.74	4.49	1.10	2.29	0.627	1.39	0.414
GLU	27.90	7.13	25.00	6.11	31.24	8.56	31.32	9.35
PRO	18.33	4.68	23.31	5.69	25.09	6.87	28.10	8.38
GLY	6.01	1.54	4.83	1.18	3.55	0.745	3.08	0.916
ALA	3.91	1.00	4.09	1.00	3.65	1.00	3.35	1.00
CYS	0.976	0.250	0.00	0.00	0.00	0.00	0.00	0.00
VAL	4.36	1.11	4.70	1.14	4.06	1.11	4.27	1.27
MET	1.35	0.346	1.48	0.362	1.23	0.336	0.912	0.272
ILEU	3.68	0.939	4.37	1.06	3.94	1.08	3.96	1.18
LEU	7.48	1.91	8.84	2.16	8.80	2.41	9.21	2.74
TYR	2.49	0.638	2.19	0.535	1.78	0.486	1.49	0.436
PHE	4.35	1.11	5.24	1.28	5.40	1.48	5.46	1.62
LAL	0.00	0.00	0.654	0.160	0.634	0.174	0.611	0.188
HIS	1.87	0.477	1.88	0.459	1.63	0.446	1.80	0.450
LYS	1.33	0.340	0.943	0.230	0.857	0.234	0.900	0.269
ARG	2.75	0.703	2.35	0.574	1.72	0.470	0.990	0.295

Table 3

Effect of alkali-treatment on amino acid composition of wheat gluten.

Conditions: 10% wheat gluten in 1N NaOH; 65°C.

A columns are mole per cent and B columns are mole ratios to alanine.

Amino Acid	Gluten Control		Time of Treatment					
			1 HR		3 HR		8 HR	
	A	B	A	B	A	B	A	B
ASP	3.20	0.819	2.69	0.796	3.02	0.805	2.84	0.807
THRE	3.19	0.816	2.09	0.619	1.65	0.440	0.983	0.279
SER	6.18	1.74	3.80	1.13	2.52	0.671	1.53	0.500
GLU	27.90	7.13	37.10	10.9	35.70	9.52	39.47	1.1
PRO	18.33	4.68	19.65	5.82	19.75	5.26	21.14	5.99
GLY	6.01	1.54	4.52	1.34	3.94	1.10	3.59	1.02
ALA	3.91	1.00	3.38	1.00	3.75	1.00	3.52	1.00
CYS	0.976	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VAL	4.36	1.11	3.93	1.16	4.24	1.13	4.02	1.14
MET	1.35	0.346	1.10	0.326	1.16	0.309	1.14	0.323
ILEU	3.68	0.939	3.42	1.01	4.11	1.09	3.40	0.965
LEU	7.48	1.91	7.19	2.13	8.30	2.21	8.29	2.35
TYR	2.49	0.638	1.94	0.574	2.06	0.550	1.58	0.450
PHE	4.35	1.11	4.41	1.30	4.64	1.23	4.50	1.27
LAL	0.00	0.00	0.493	0.146	0.835	0.222	0.610	0.173
HIS	1.87	0.477	1.68	0.498	1.61	0.428	1.45	0.410
LYS	1.33	0.340	0.774	0.195	0.846	0.225	0.846	0.240
ARG	2.75	0.703	1.83	0.541	1.85	0.495	1.06	0.300

do not vary significantly in a 1% or 10% gluten suspensions at high pH. However, protein concentration does seem to influence the extent of LAL formation at lower pH (2), presumably because NH_2 addition to dehydroalanine is rate-limiting.

The observed absence of a concentration effect can probably be rationalized as follows with the aid of Figures 3-5. Since elimination reactions of serine, threonine, and cystine to form dehydroalanine side chains are second-order reactions that depend on the concentration of both hydroxide ion and susceptible amino acid side chain, the extent of crosslinking should be a function of hydroxide ion concentration. This is indeed the case (24). However, because the reaction of the ε-amino group of lysine with a vinyl-type compound such as dehydroalanine is also a second-order reaction (25, 26), it would have been expected that an increase of protein concentration should lead to a corresponding increase in rate of formation of lysinoalanine. Since this apparently does not occur, the major factor controlling formation of lysinoalanine, once the rate-determining dehydroalanine precursors are formed, may be the location of required partners for crosslink formation. Only dehydroalanine and lysine residues situated on the same or closely adjacent protein chains are favorably placed to form crosslinks. Once the convenient sites are used up, additional lysinoalanine or other crosslinks form less readily. Each protein, therefore, may have a limited fraction of potential sites for "productive" formation of crosslinked residues. The other sites are "unproductive" in the sense discussed. The number of such sites may vary and is presumably dictated by the protein's size, composition, conformation, chain mobility, and steric factors.

As already mentioned, the rates of both formation and destruction of lysinoalanine may be greater in more concentrated solutions, accounting for the observed lysinoalanine content, which appears to be essentially independent of initial protein concentration for wheat gluten suspensions. In addition, since the rate of lysinoalanine formation is pH-dependent (Table 1), any buffering effect of the protein, which should be greater at the higher concentrations,may also influence the extent of lysinoalanine formation.

Effect of Acylation. Since formation of lysinoalanine from lysine requires the participation of an ε-amino group of a lysine side chain, it was expected that protection of amino groups by acylation (acetylation, succinylation, etc.), as illustrated in Figures 6 and 7 should minimize or prevent lysinoalanine formation under alkaline conditions, if the protective gorup(s) survive the treatment. This expectation was indeed realized. Results in Table 4 strikingly demonstrate that both acetylation and succinylation of wheat gluten prevent lysinoalanine formation. Although acylation also prevented the destruction of lysine, the other amino acids (cystine, serine, threonine, arginine, and, to some extent, tyrosine) were modified.

Analogous results were noted when acetylated and succinylated soy protein (promine-D) were subjected to alkaline treatment.

As previously noted, modifications of proteins with acetic anhydride has limited application to alter the functionality of proteins for various purposes (16). Acetic anhydride transforms basic amino groups to neutral amide side chains. In contrast, succinylation introduces a four-carbon side chain with a negatively charged carboxyl group. Since such negatively charged carboxyl groups electrostatically repel negatively charged hydroxyl groups of the

Table 4

Effect of acetylation and succinylation on amino acid composition of alkali-treated wheat gluten. Numbers are mole per cent.

Amino Acid	Gluten Control (untreated)	10% Acetylated gluten +NaOH 1 Hr	1% Acetylated gluten + NaOH 3 Hrs	10% Acetylated gluten + NaOH 3 Hrs	1% Succinylated gluten + NaOH 1 Hr
ASP	3.20	3.16	3.37	3.38	3.13
THRE	3.19	2.18	1.32	1.55	2.17
SER	6.81	4.31	2.64	2.85	4.19
GLU	27.90	35.42	30.58	35.77	35.45
PRO	18.33	16.42	20.43	16.94	16.46
GLY	6.01	5.30	4.79	4.89	5.37
ALA	3.91	3.96	4.02	4.21	3.92
VAL	4.36	4.27	4.74	4.59	4.43
MET	1.35	1.35	1.39	1.36	0.737
ILEU	3.68	3.59	4.26	3.83	3.92
LEU	7.48	7.68	9.41	8.38	8.11
TYR	2.49	2.23	2.14	2.03	2.44
PHE	4.35	4.59	5.75	4.63	4.98
LAL	0.00	0.00	0.00	0.126	0.00
HIS	1.87	1.75	1.76	1.79	1.77
LYS	1.33	1.39	1.54	1.59	1.14
ARG	2.75	2.37	1.82	2.02	1.77

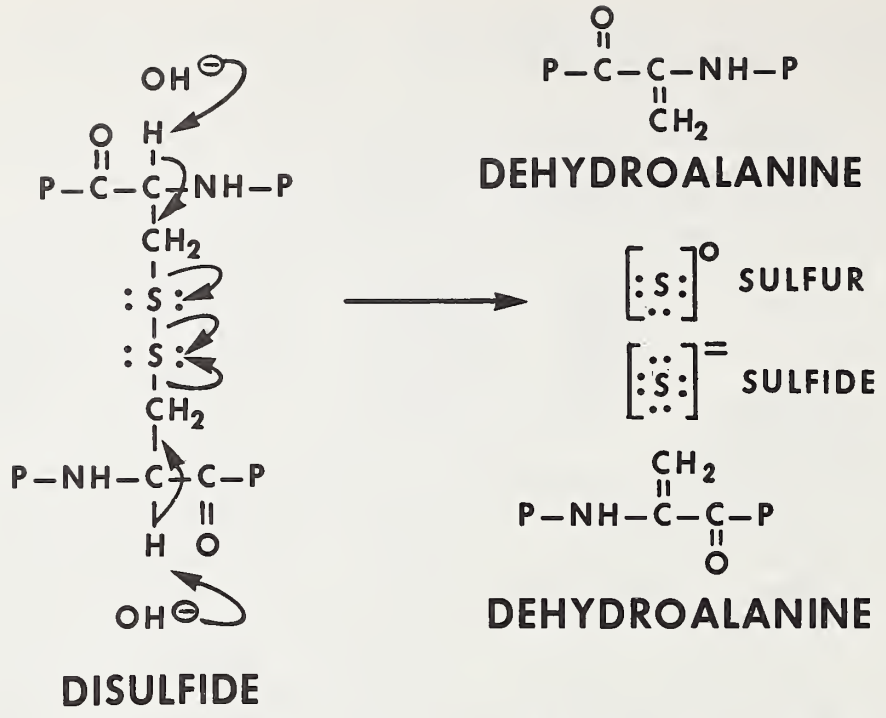

Figure 3. Postulated mechanism for base-catalyzed transformation of a protein-disulfide bond to two dehydroalanine side chains, a sulfide ion, and sulfur.

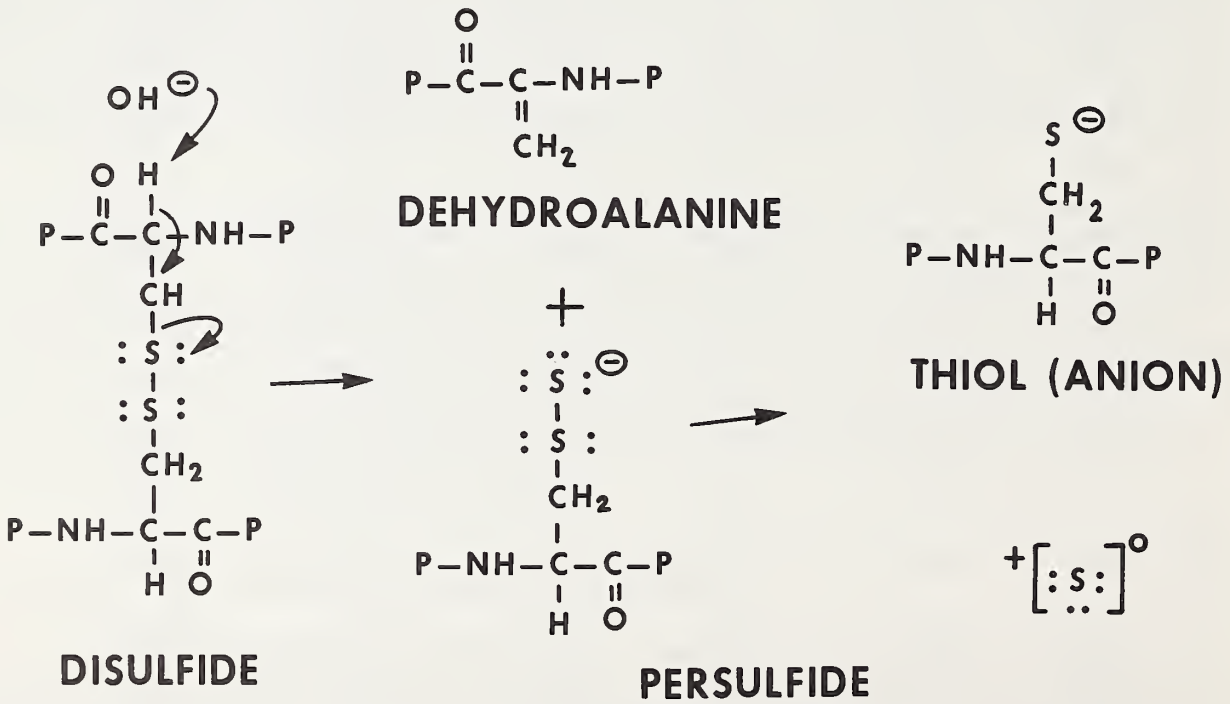

Figure 4. Postulated mechanism for base-catalyzed formation of one dehydroalanine side chain and one persulfide anion from a protein disulfide bond. The persulfide can decompose to a thiol anion (cysteine) and elemental sulfur.

MECHANISM OF LYSINOALANINE FORMATION

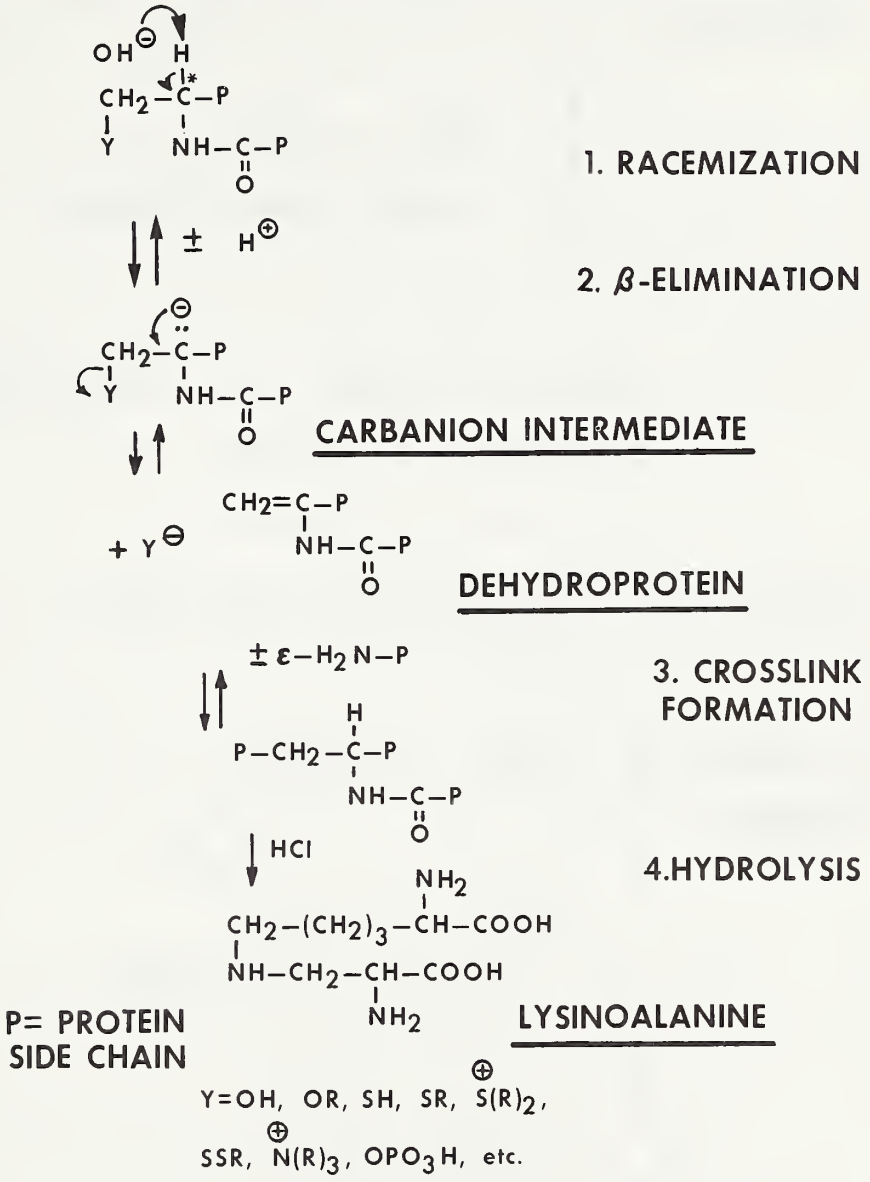

Figure 5. Transformation of a reactive protein side chain to a lysinoalanine side chain via elimination and crosslink formation. Note that the intermediate carbanion has lost the original asymmetry of the reactive amino acid side chain. The carbanion can combine with a proton to regenerate the original amino acid side chain which is now racemic or undergo an elimination reaction to form dehydrolanine.

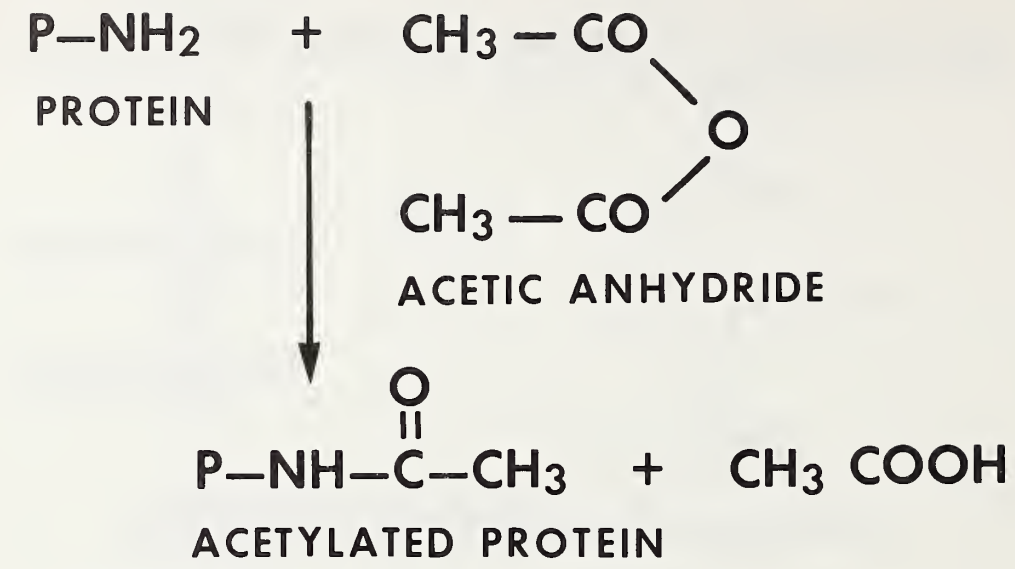

Figure 6. Acetylation of a protein amino group by acetic anhydride.

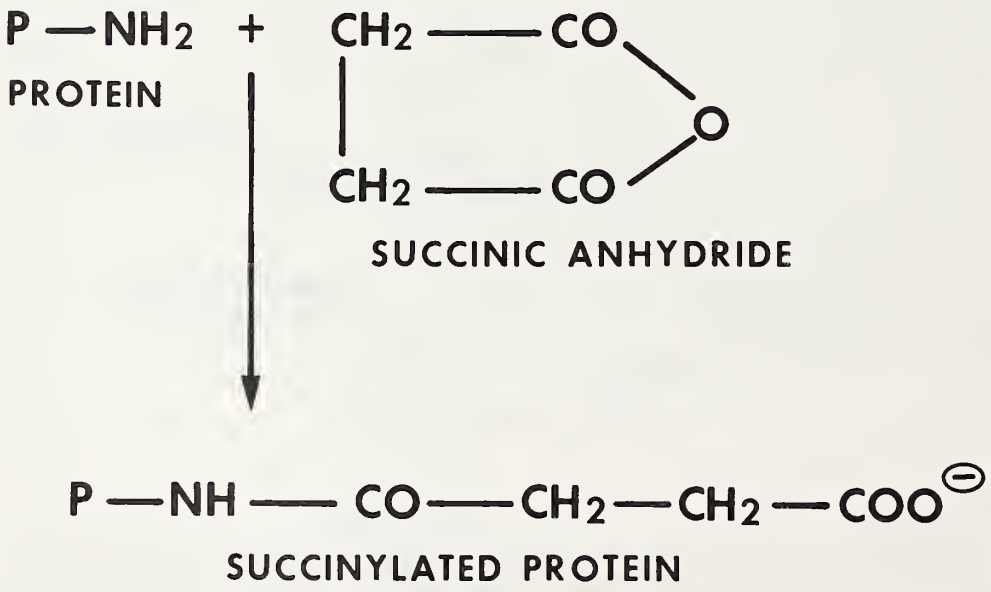

Figure 7. Succinylation of a protein amino group by succinic anhydride.

alkaline medium, it was expected that succinylation should not only prevent lysinoalanine formation but also minimize elimination reactions leading to the destruction of serine, threonine, and cystine. The data in Table 4 do not support this expectation. Possibly the repulsion of carboxylate ions causes unfolding of side chains making the protein more accessible to alkali attack (27, 28). Also, the ionic strength of the medium may be so high that the local charges are screened from one another by the ion atmosphere.

Finally, if acylated proteins turn out to be as nutritionally available and safe as unmodified proteins, then acylation may be of practical value to protect proteins against formation of lysinoalanine. It should be noted, however, that conditions described in this paper are more severe than generally used in food processing. Since commercial alkali-treatment of proteins is mostly carried out above pH 10 at room temperature for short periods, the actual lysinoalanine content of most alkali-treated foods is probably small. It should be emphasized,however, that significant amounts of lysinoalanine appear to be formed when wheat gluten is treated at pH 10.6 at 65°C (Table 1). Consequently, preventive measures may be justified even if proteins are subjected to alkaline conditions in the pH region 10 to 11.

Mechanisms of Inhibition of Lysinoalanine Formation

Lysinoalanine and related crosslinked amino acids may be derived from reaction of lysine with dehydroalanine residues formed by elimination reactions from serine, cystine, and possibly cysteine residues in proteins. Threonine residues react similarly to form methylated homologues (8). The double bond of dehydroalanine, which is part of an activated, conjugated double-bond system, reacts readily with SH and NH_2 groups of cysteine and lysine and possibly also with NH groups of histidine side chains to form lanthionine, lysinoalanine, and possibly also histidinoalanine (8). The relative rates of these reactions and the accessability of reactive sites to one another appear to determine the nature of heated or alkali-treated food proteins.

Inhibition of lysinoalanine formation by added sulfite ions or thiols can occur by at least three distinct mechanisms (8, 16). First, by direct competition the added nucleophile (mercaptide, sulfite, bisulfite, thiocyanate, thiourea, etc.) can trap dehydroalanine residues derived from protein amino acid side chains to form their respective adducts. In particular, lanthionine side chains, illustrated in Figures 8 and 9,are formed from added cysteine and N-acetyl-cysteine (direct competition mechanism). Second, the added nucleophile can cleave protein disulfide bonds and thus generate free protein SH groups, which, may, in turn, combine with dehydroalanine residues, as illustrated in Figure 10 (indirect competition mechanism). Third, the added nucleophile, by cleaving disulfide bonds, can diminish a potential source of dehydroalanine, inasmuch as the resulting cysteine residues undergo elimination reactions to form dehydroalanine much less readily than the original cystine (disulfide) precursor residues (suppression of dehydroalanine formation mechanism, Figure 11). Generally, it would be predicted that negatively charged protein side chains such as sulfite ($P\text{-}S^-$), persulfide ($P\text{-}S\text{-}S^-$), sulfite ($P\text{-}SO_3^-$), thiosulfate ($P\text{-}S\text{-}SO_3^-$), etc., will undergo base-catalyzed elimination more slowly than neutral disulfide bonds because the negative charge would repel

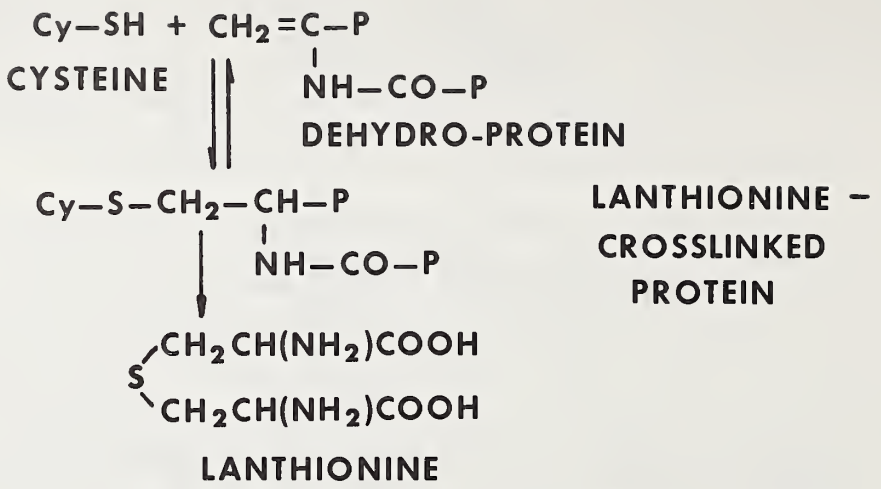

Figure 8. Inhibition of lysinoalanine (crosslink) formation by added cysteine, which combines at a faster rate with the double bond of a dehydro-protein to form a lanthionine crosslink than does the amino group of a lysine residue to form a lysinoalanine crosslink.

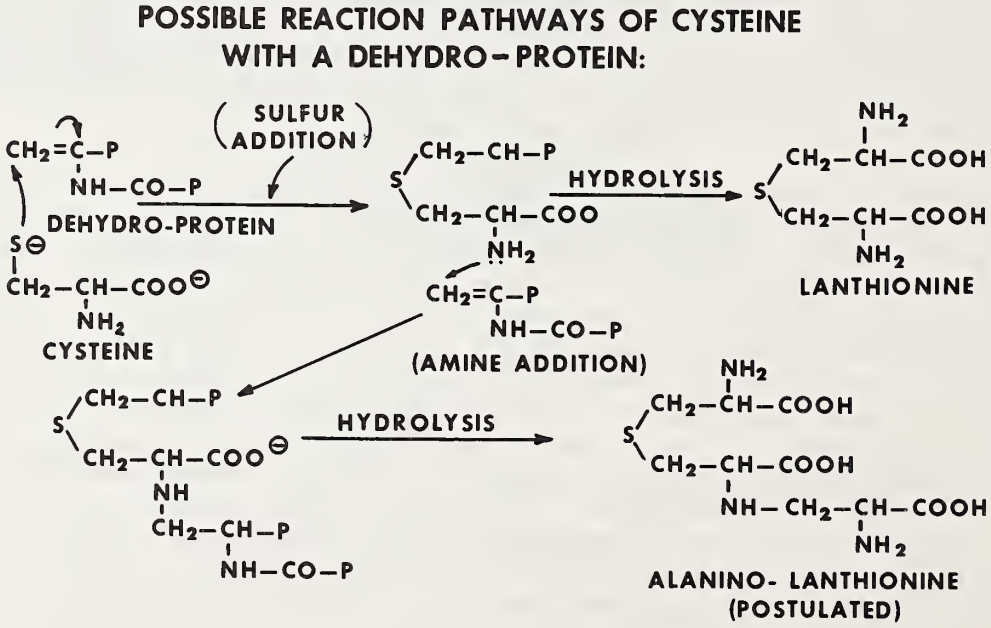

Figure 9. Competitive reactions of the ionized sulfhydryl and amino groups of cysteine with the double bond of dehydroalanine.

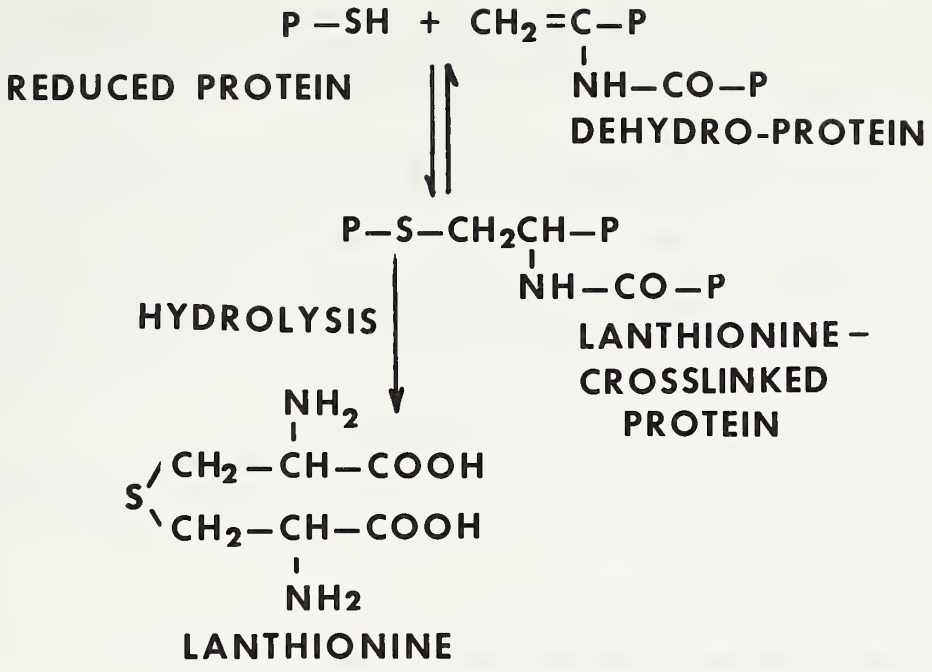

Figure 10. Inhibition of lysinoalanine formation by indirect competion where the SH group of a reduced protein combines with the double bond of a dehydro-protein, thus preventing it from reacting with the amino group of lysine to form lysinoalanine.

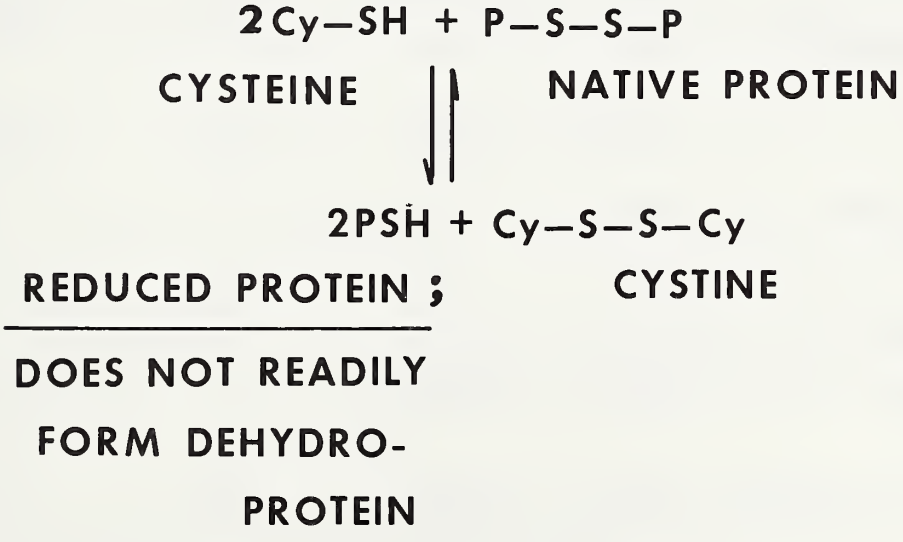

Figure 11. Inhibition of lysinoalanine by suppression of dehydro-protein formation. The added cysteine can perform two functions. It can combine with a dehydro-protein (Fig. 8) and/or reduce a protein disulfide.

the negatively charged hydroxide (OH^-) ions. In fact, the presence of sulfite ions (8) and of thiol derivatives such as cysteine (2, 13) has been shown to decrease the amount of lysinoalanine formed during alkali-treatment of several proteins and wool.

To gain further insight into the nature of the protective effect of such external additives and to evaluate the relative effectivness of structurally different organic and inorganic nucleophiles, the effects of several organic and inorganic compounds on the lysinoalanine and lysine contents of alkali-treated wheat gluten are compared in Table 5. These preliminary results show that all these compounds partly inhibit lysinoalanine formation. The extent of inhibition may vary from protein to protein and should be related to both the content and reducibility of the disulfide bonds. In the case of wheat gluten, acylation by succinic or acetic anhydrides, rather than adding the external nucleophiles during the alkaline treatment, appears more effective for preventing lysinoalanine formation. Moreover, cleavage of disulfide bonds by added sulfide, sulfide, bisulfite, etc. destroyes the original structural integrity of the protein.

Table 5

Effect of additives on lysine and lysinoalanine content of wheat gluten. Conditions: 1% wheat gluten (1g/100cc) in 1N NaOH; 1 hour; 65°C. A columns are mole per cent and B columns mole ratios to alanine.

Compound added	A		B	
	Lysine	Lysinoalanine	Lysine	Lysinoalanine
Untreated gluten control	1.33	0.00	0.340	0.00
None	0.913	0.654	0.230	0.160
sodium sulfite (200 mg)	1.23	0.552	0.273	0.123
sodium bisulfite (200 mg)	0.733	0.253	0.210	0.072
L-cysteine (50 mg)	0.987	0.263	0.272	0.073
N-acetyl-L-cysteine (50 mg)	0.933	0.304	0.258	0.084
Thiourea (200 mg)	0.979	0.363	0.261	0.097

A final word of caution is in order. Shapiro and Gazit (29) have shown that bisulfite ions can crosslink nucleic acids and proteins, and may, therefore, be mutagenic. Furquharson and Adams (30) have shown that vitamin B_{12} (aquocobalamin) can react with bisulfite ions *in vitro* to form sulfitocobalamin, presumably inactivating the vitamin. Consequently, use of such additives in foods and feeds is inadvisable without prior detailed nutritional and toxicological studies (31).

Related chemical and photochemical transformations of wheat gluten, including reactions in nonaqueous solvents such as dimethyl sulfoxide and in the presence of strong bases such as sodium and sodium hydride, are described elsewhere (23, 32-42).

Acknowledgments

It is a pleasure to thank Linda Loyd and Eddie Marshall for excellent technical assistance and Amy T. Noma for her help with the amino acid analyses.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which also may be suitable.

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GLUTENIN: STRUCTURE AND FUNCTIONALITY IN BREADMAKING

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INTRODUCTION

According to the Osborne classification of wheat endosperm proteins, glutenin is that fraction that remains in the residue after sequential extraction of flour with dilute sodium chloride and 70% ethanol solutions. It comprises approximately 40% of the protein of bread wheat flour. It is the protein that contributes elasticity to gluten and dough, and thereby, plays a key role in the breadmaking properties of a wheat flour. This article reviews recent studies from our and other laboratories on the structure of glutenin and its functionality in breadmaking.

SOLUBILITY AND BREADMAKING

Glutenin appears to be directly involved in dough development and breakdown during mixing. Wheat flours that require long mixing for optimum dough development contain a relatively higher proportion of glutenin that is unextractable (insoluble glutenin) by 0.1N acetic acid solution after the albumins, globulins, and gliadins are extracted from the flour (Orth and Bushuk, 1972; Orth et al, 1972; Bietz and Wall, 1975). Gel-filtration results indicate that the glutenin fraction of wheats with longer dough development times has a higher average molecular weight (MW) than the glutenin fraction of short mixing flours (Tanaka and Bushuk, 1973b; Huebner and Wall, 1976). During dough mixing, insoluble glutenin is gradually converted into the acetic acid-soluble glutenin. The rate of this "solubilization" of glutenin is faster in doughs of weaker flours and is accentuated by fast acting thiol oxidizing agents such as potassium iodate and thiol blocking agents such as N-ethylmaleimide (Tanaka and Bushuk, 1973a). Evidence from disc-gel electrophoresis suggests that glutenin either depolymerizes or disaggregates into subunits during dough mixing (Tsen, 1967; Tanaka and Bushuk, 1973b,c).

It is well known that for a single wheat variety or a class of similar varieties, loaf volume is directly proportional to the protein. However, the slope of the loaf volume-protein content relationship varies among varieties. There is some disagreement as to which protein fraction is responsible for this intervarietal variation.

One of the first reports on the functionality of glutenin in breadmaking was that of Pomeranz (1965). He reported that the proportion of flour protein that could be extracted (solubilized) with 3M urea solutions (albumin, globulin, gliadin and soluble glutenin) was inversely related to baking quality. Conversely, the proportion of the protein that was insoluble in 3M urea was directly related to loaf volume. These findings were later confirmed by baking studies on reconstituted flours (Shogren et al, 1969).

A comprehensive study of the relationship between the protein fractions obtained by the Osborne solubility fractionation and baking quality was

published from our laboratory (Orth and Bushuk, 1972; Orth et al, 1972). This study of 26 widely different spring wheat varieties grown at four locations in Western Canada showed that loaf volume was inversely related to the proportion of the soluble (in 0.1N acetic acid) glutenin (Fig. 1) and directly related to the proportion of insoluble glutenin (Fig. 2). These relationships were not affected by environment and therefore can be considered to be one of the inherited characteristics that determines baking quality. The variations in the proportions of albumins, globulins, and gliadins for the 26 varieties were not statistically significant. On the basis of these statistical correlations, Orth and Bushuk (1972) concluded that glutenin is responsible for the variations in loaf volume (at constant protein content) among varieties of bread wheat. This conclusion differed from that of Hosenay et al (1969) who on the basis of their experiments had concluded that the gliadin proteins control the loaf-volume potential of a wheat flour. This apparent controversy remains unresolved.

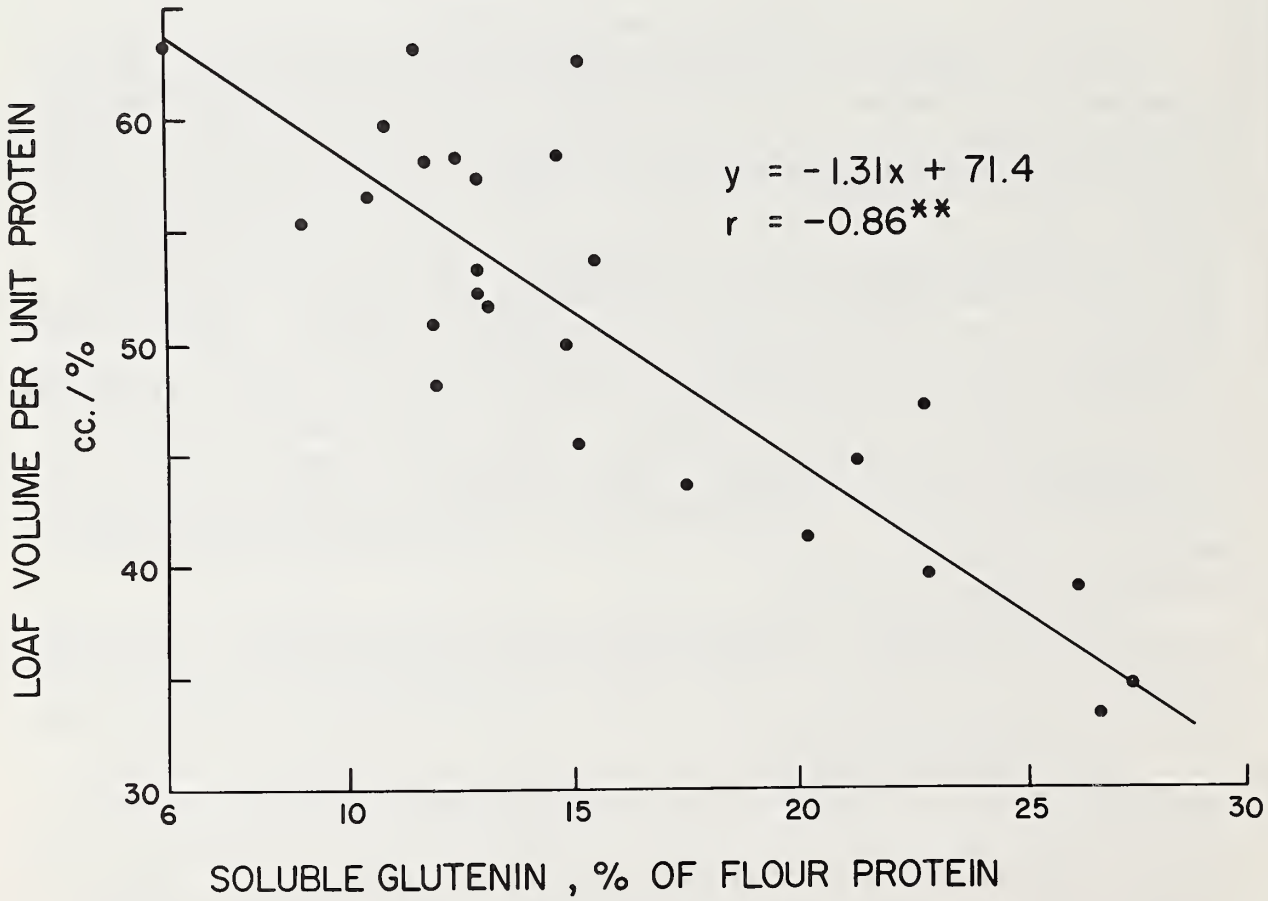

Figure 1. Relationship between loaf volume per unit protein content and the proportion of soluble glutenin in the flour protein of 26 common wheat varieties.

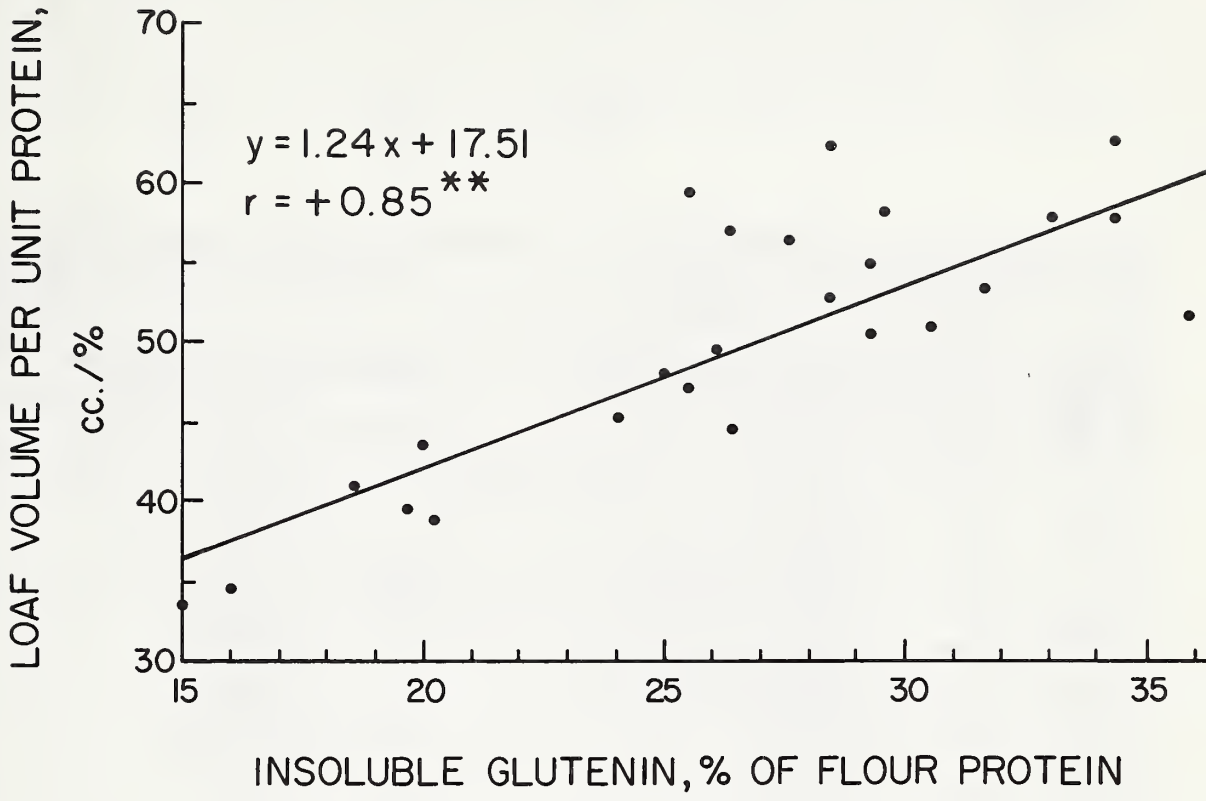

Figure 2. Relationship between loaf volume per unit protein content and the proportion of insoluble glutenin in the flour protein of the 26 common wheat varieties.

STRUCTURE

Physicochemical Studies

The key to the functional behavior of glutenin lies in its physical (molecular size, shape and tendency to aggregate) and chemical (amino acid composition and sequence) properties.

There have been numerous attempts to determine the molecular weight (MW) of glutenin. Average MW's reported in the literature range from 150,000 to 3 million (Jones et al, 1961; Nielson et al, 1962; Taylor and Cluskey, 1962). Gel-filtration on Sephadex G-150 (Fig. 3) of the total flour protein extracted by a highly dissociating solvent containing acetic acid, urea, cetyltrimethylammonium bromide (AUC) showed that only a small fraction of the glutenin dissolved in AUC elutes in the 100,000 to 150,000 MW range; the remainder elutes in the void volume (Bushuk and Wrigley, 1971).

Although the MW of glutenin has not been accurately determined, there is considerable evidence which indicates that this protein is extremely polydisperse and that the MW distribution may be a varietal characteristic

that is important to breadmaking quality. The rheological properties of glutenin, hence gluten and dough, could derive directly from the MW distribution. This could be one source of varietal differences in breadmaking quality.

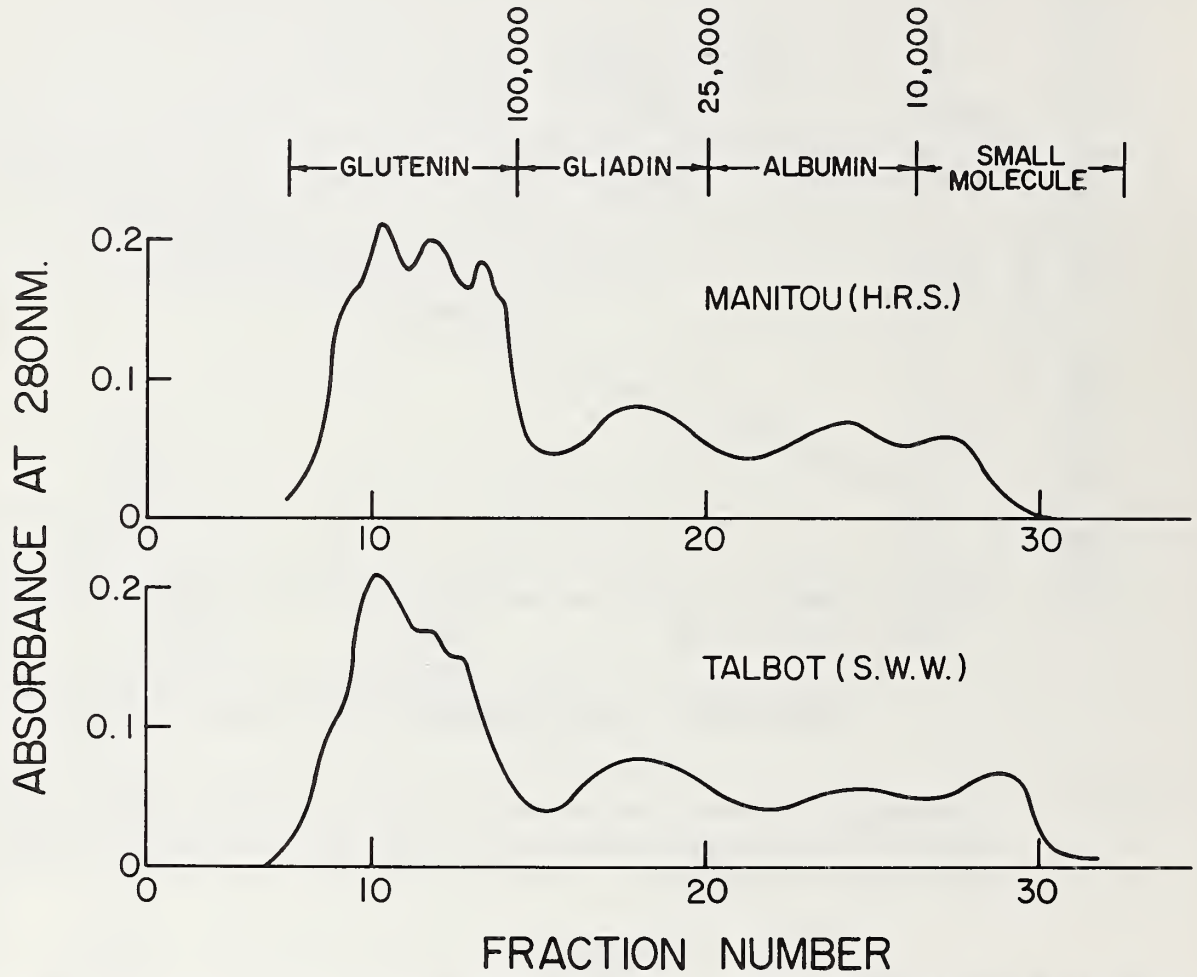

Figure 3. Gel-filtration on Sephadex G-150 of flour proteins extracted with acetic acid-urea-cetyltrimethylammonium bromide (AUC) solvent from two common wheat varieties of widely different breadmaking quality (Manitou, good quality variety; Talbot, poor quality variety).

By contrast, the gliadin proteins have a relatively narrow MW distribution, from about 20,000 to 50,000 (Jones et al, 1961).

The idea that glutenin molecules consist of polypeptide subunits held together by disulfide bonds followed from the work of Pence and Olcott (1952) on the effect of disulfide reducing agents on the viscosity of gluten. Subsequently, Woychik et al (1964) showed, by starch-gel electrophoresis, that some of the subunits obtained by reducing glutenin, resembled gliadin. However, the glutenin also contained subunits that were different from gliadin, indicating that it is not a polymer of gliadin as was suspected at that time.

More convincing evidence for the uniqueness of glutenin came from

experiments in which reduced glutenin was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was first applied to glutenin by Bietz and Wall (1972) and later by Orth and Bushuk (1973a). The technique separates a highly complex mixture of proteins according to MW. SDS-PAGE showed that reduced glutenin was a highly complex mixture of subunits ranging in MW from 12,000 to 134,000. Figure 4 shows typical patterns of reduced glutenin from bread and durum wheats. Bread wheat glutenin contains approximately 17 subunits while durum wheat glutenin contains approximately 15 subunits. Durum wheats lack two of the large subunits of glutenin present in bread wheats.

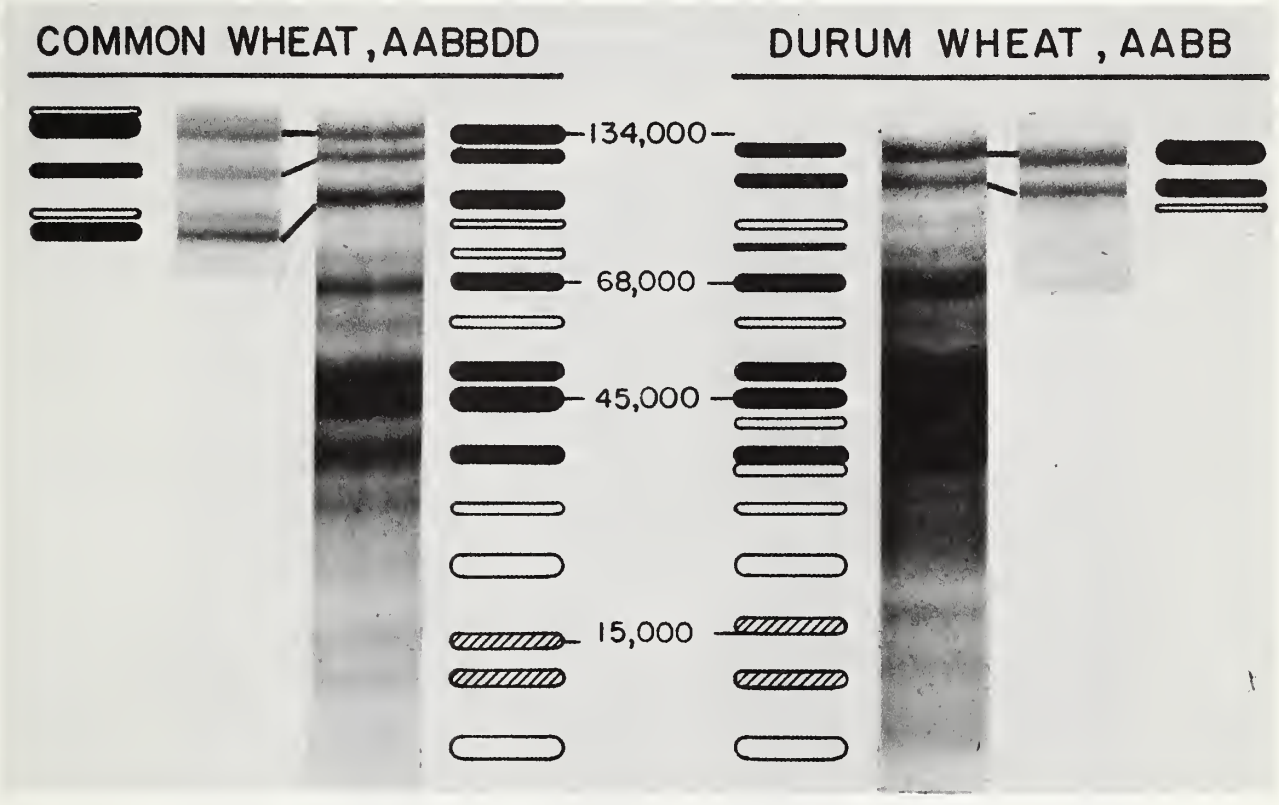

Figure 4. Typical SDS-PAGE patterns of reduced glutenins of bread and durum wheats.

Further evidence for the complexity and uniqueness of glutenin came from the work of Huebner and Wall (1974), and Khan (1977). These workers subjected reduced and alkylated glutenin to gel-filtration on Sephadex G-200 and obtained three distinct protein peaks (Fig. 5). Peak I eluted with the void volume but, by SDS-PAGE, it contained subunits with MW (by SDS-PAGE) of 68,000 to 12,000. Peak I subunits, therefore, exhibited a strong tendency to aggregate, a phenomenon which might be important in the functionality of

glutenin in breadmaking. Peak II contained the largest subunits of glutenin while peak III contained those subunits with the same mobility by SDS-PAGE as the two major gliadin proteins (MW 35,000 and 45,000). Gel-filtration results showed that glutenin is composed of three distinct groups of subunits, each group, perhaps contributing its unique properties to the functional properties of glutenin.

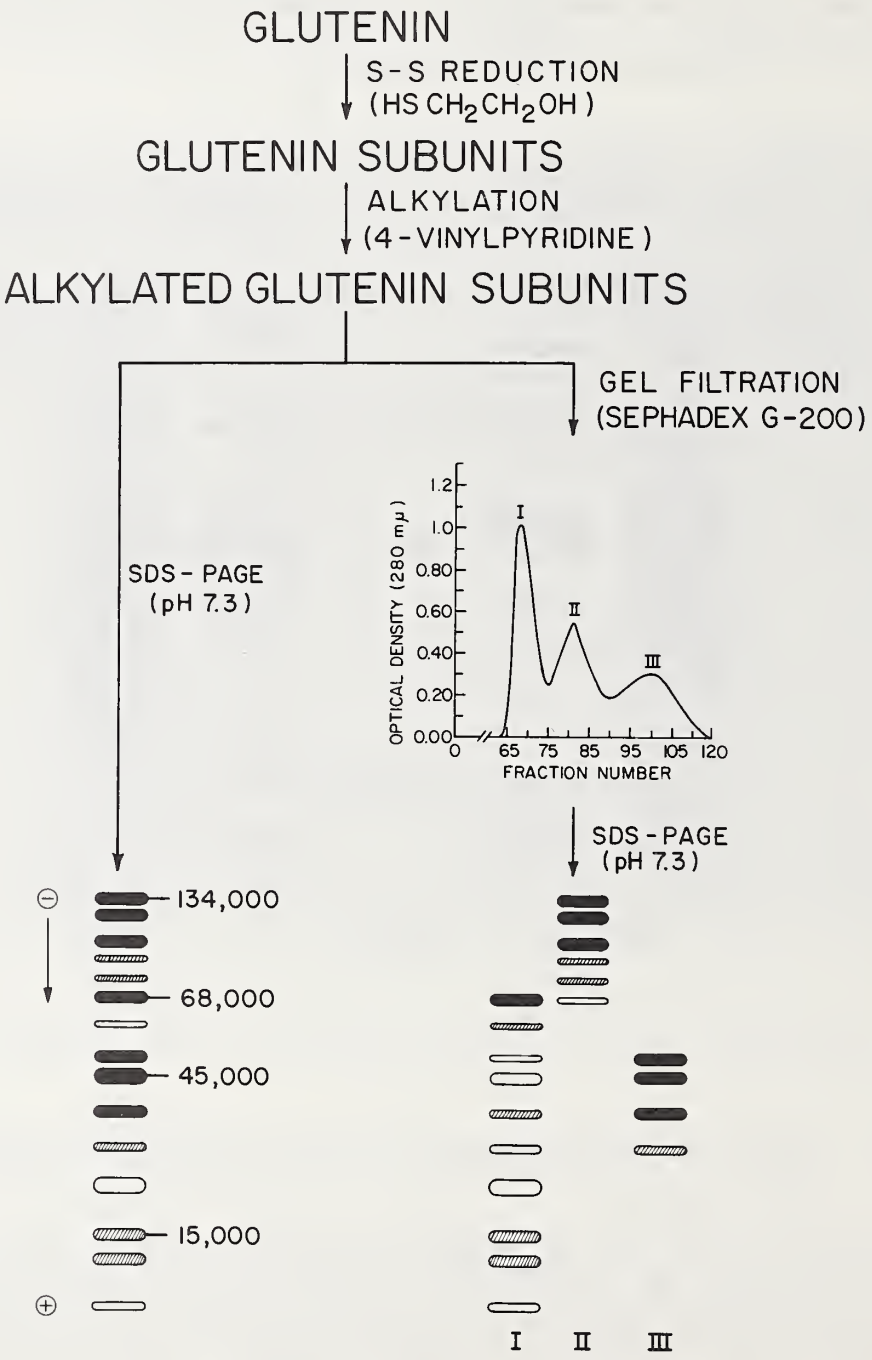

Figure 5. SDS-PAGE of alkylated glutenin subunits of bread wheat fractionated gel-filtration on Sephadex G-200.

Further evidence on the complexity of glutenin came from the analysis by SDS-PAGE of unreduced glutenin by Khan (1977). He showed that unreduced acetic acid-soluble glutenin from the Osborne solubility fractionation procedure, even after extensive purification, contained many prominent protein components that entered the SDS-gel (Fig. 6A). On the other hand, the insoluble glutenin (residue proteins from Osborne procedure), when subjected to SDS-PAGE in the unreduced state, showed fewer and much fainter protein components that entered the SDS-gel (Fig. 6B). These protein components from the unreduced acetic acid-soluble and insoluble glutenins that enter the SDS-gel correspond in mobility to many of the subunits of reduced glutenin in the MW region of 12,000 to 68,000. The first five high MW subunits of reduced glutenin are not seen in the SDS-PAGE patterns of unreduced glutenin. Therefore, it seems that the subunits with MW of 12,000 to 68,000 are associated through non-covalent forces to form the functional glutenin aggregate.

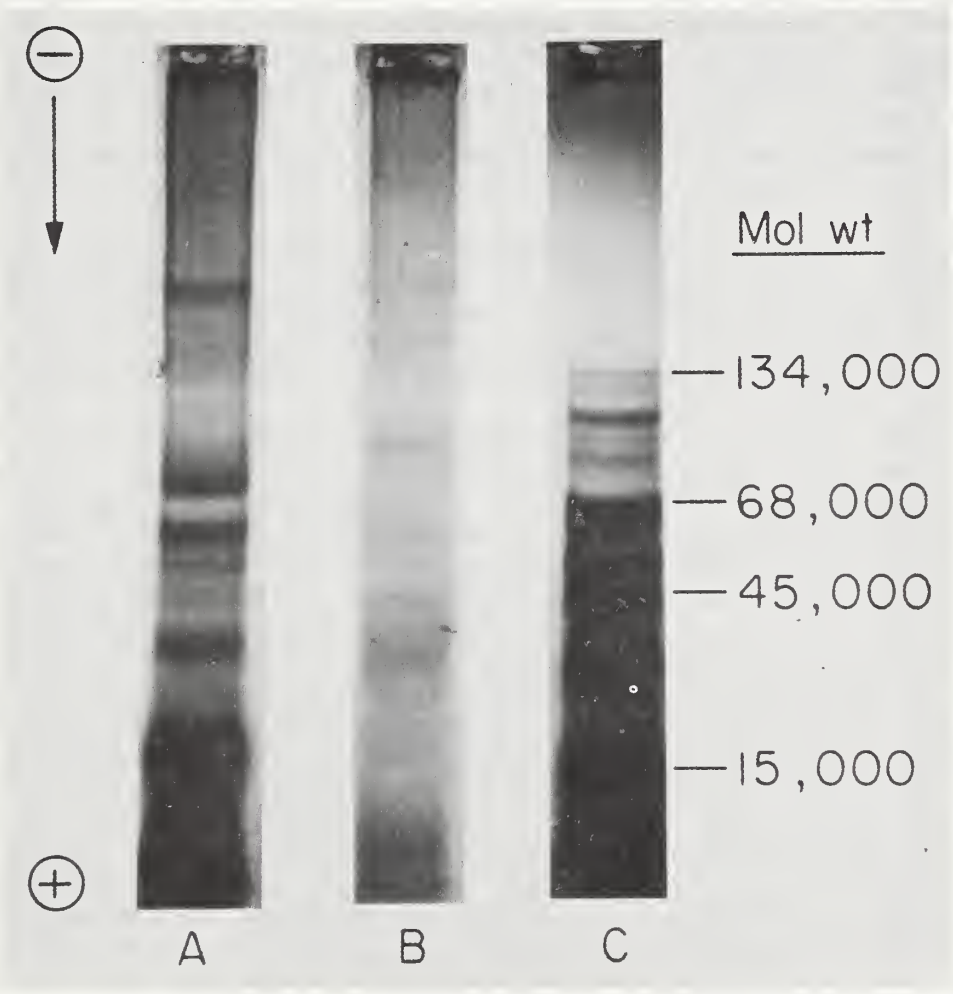

Figure 6. SDS-PAGE patterns of unreduced acetic acid-soluble and insoluble glutenins and reduced "total" glutenin (A, B and C, respectively).

In addition to the protein components that enter the SDS-gel from unreduced glutenin, there is considerable protein that remains at the point of

sample application (origin of gel patterns). There was more protein at the origin of the patterns of the insoluble glutenin than in those of the soluble glutenin. Solubility fractionation (Orth and Bushuk, 1972; Orth et al, 1972; Pomeranz, 1965) and baking studies (Shogren et al, 1969) have been used to show that the acetic acid-soluble glutenin was negatively correlated to breadmaking quality (loaf volume) while the insoluble glutenin was positively correlated to breadmaking quality. Our recent results (Khan, 1977) have raised another question; what is the functional importance of the two types of proteins that make up unreduced glutenin (those that enter the SDS-gel and those that remain at the point of sample application)? In the discussion that follows, the proteins that enter the SDS-gel from unreduced glutenin will be referred to as glutenin I (Glu I) and those that remain at the point of sample application as glutenin II (Glu II). These two designations will be used later to describe a model of functional glutenin.

The amino acid composition of glutenin shows that approximately every third residue is glutamine (Ewart, 1967). Thus, there are numerous amide groups that can form inter- and intra-molecular hydrogen bonds. This extensive hydrogen bonding is a very important feature of the physical (rheological) properties of hydrated glutenin. Glutenin, together with gliadin, are examples of proteins whereby the natural selection in the evolution of wheat, based on an efficient storage of nitrogen for the new plant, has produced a protein with extremely useful functional properties for breadmaking.

Glutenin also contains a relatively high proportion of hydrophobic amino aicds such as leucine. The non-polar side chain of leucine can interact with each other, especially in an aqueous environment, to form the so-called hydrophobic bond. A large number of these relatively weak bonds, acting collectively, can contribute substantially to stabilizing glutenin aggregates (Chen et al, 1975).

Glutenin contains relatively small proportions of amino acids with acidic or basic side groups, hence its poor solubility in aqueous solvents.

A unique feature of the amino acid composition of the high MW subunits of glutenin (e.g. Peak II of Fig. 5) is the relatively high contents of glycine, proline, glutamine and leucine (Table 1). Glycine is present in high proportion in structural proteins such as collagen. Proline forms kinks or bends wherever it is found in a polypeptide chain, thereby disrupting the regular secondary structure. The high content of glutamine is necessary for hydrogen bonding while leucine promotes hydrophobic interactions. These amino acids, therefore, may be extremely important in determining the physical structure (hence functionality) of glutenin. Viscosity studies of glutenin dispersions indicate a very flexible asymetric tertiary structure (Taylor and Cluskey, 1962). Most of the polypeptide chains of glutenin form random coils and only small portions have helical structures (Wu et al, 1967). The conformation that native glutenin assumes may be stronly dependent on the unique amino acid composition and sequence of the high MW subunits.

Model of Functional Glutenin

On the basis of information available at the present time, it is not possible to develop a detailed model of the molecular structure of glutenin. However, a number of working models have been postulated in an attempt to explain the rheological properties of glutenin, gluten and dough (Ewart, 1968,

Table 1. Amino acid composition[1] of glutenin and the protein fractions obtained from gel-filtration of reduced and alkylated[2] glutenin (see Fig. 5)

Amino acid[3]	Glutenin	Peak I	Peak II	Peak III
Lysine	1.91	3.81	0.73	0.83
Histidine	1.66	2.02	0.73	1.59
Arginine	4.01	4.76	2.14	3.66
Aspartic acid	3.48	7.63	0.69	2.32
Threonine	3.20	4.06	3.03	2.66
Serine	6.26	6.45	6.34	6.49
Glutamic acid	32.24	19.99	40.42	39.32
Proline	12.15	7.93	12.30	14.99
Glycine	9.04	9.75	17.72	3.59
Alanine	4.06	6.96	2.79	2.72
Valine	4.10	5.84	1.58	4.25
Methionine	1.57	1.82	0.29	0.71
Isoleucine	3.01	3.92	0.87	3.67
Leucine	6.79	8.25	4.30	7.34
Tyrosine	3.04	3.41	5.41	1.42
Phenylalanine	3.43	3.39	0.63	4.40

[1]Expressed as mole percent.

[2]According to Friedman et al (J. Biol. Chem. 245, 3868, 1970).

[3]Cysteine and tryptophan were not determined.

1972, 1977; Kasarda et al, 1975). In Ewart's most recent model, the subunits of functional glutenin are joined by interpolypeptide disulfide bonds (four for each interior subunit) to form long concatenated structures. On the other hand, Kasarda et al (1975) have proposed an entirely different model for functional glutenin. In this model, there are no interpolypeptide disulfide bonds; the subunits are held by specific interacting secondary forces (hydrogen bonds, and ionic and hydrophobic interactions).

On the basis of the physicochemical data (solubility, SDS-PAGE, gel-filtration, isoelectric focusing and amino acid composition) obtained in our laboratory (Khan, 1977), we propose the model shown in Figure 7 for functional glutenin. The model incorporates the two types of glutenin I (Glu I) and glutenin II (Glu II) indicated earlier. It is postulated that the strength of the association, or conversely, the ease of extraction (e.g. by acetic acid), of Glu I from flours of different cultivars is related to the physical properties of the dough obtained from each flour. Further, it is postulated that Glu I forms the mobile linkages in gluten and dough structure between the high MW Glu II in which the subunits are joined by interpolypeptide disulfide bonds. The elastic modulus of the glutenin (hence dough) would depend on the ratio of Glu I to Glu II.

It is further postulated that Glu II is highly heterogeneous in molecular size. Glu II may be more or less compactly folded depending on the number and location of disulfide crosslinkages. The greater the amount

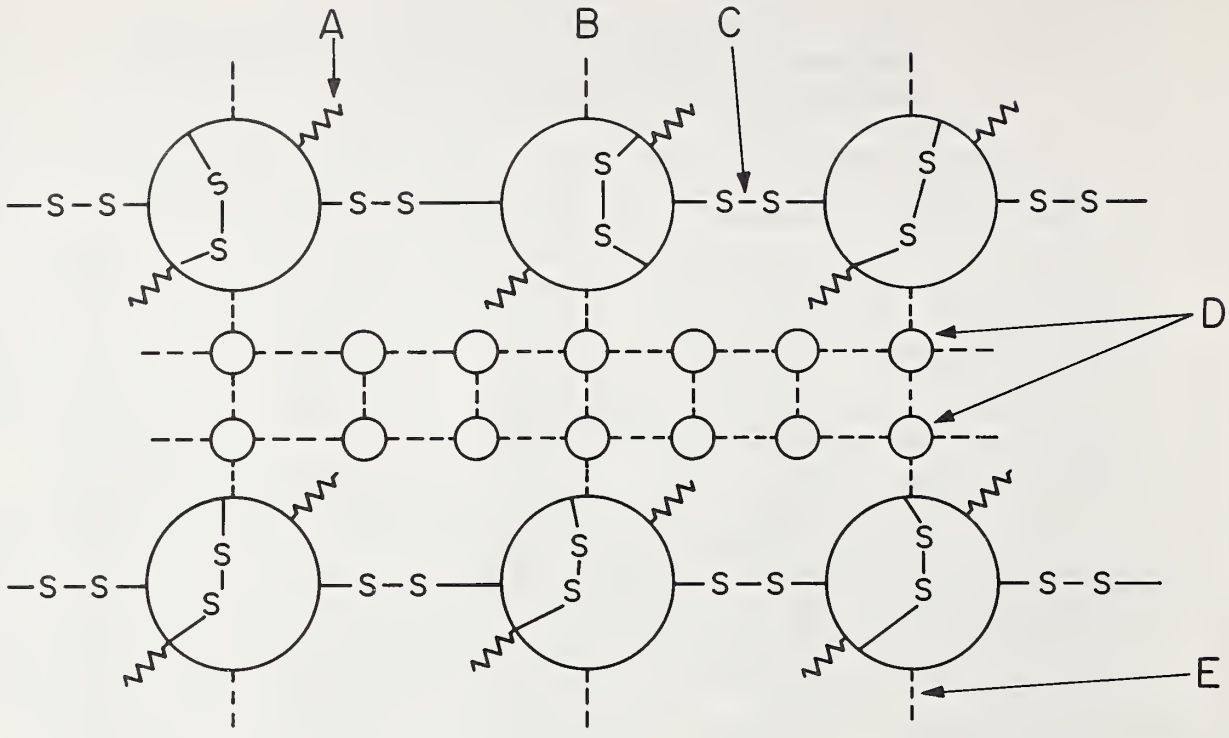

Figure 7. Schematic representation of the model of functional glutenin.

of and the more compact that Glu II is, the lesser the amount of acetic acid-soluble protein that can be solubilized from total glutenin and, therefore, the greater the amount of proteins that remain in the insoluble residue. Solubility fractionations (Orth and Bushuk, 1972; Bietz and Wall, 1975) have shown that certain cultivars have less acetic acid-soluble glutenin and more insoluble (residue) glutenin. SDS-PAGE results of this study suggest that the acetic acid-soluble glutenin is probably mainly Glu I, and hence, the residue protein (insoluble glutenin) would be mainly Glu II.

The influence of the quantity and molecular size of Glu II becomes evident during a very critical stage of the breadmaking process, dough mixing. Cultivars that contain a greater proportion of Glu II will yield doughs that are more elastic. Dough of such cultivars would require longer development times and withstand greater over-mixing (i.e. have longer mixing tolerance). Glu I forms non-covalent crosslinks among Glu II molecules. Because of the non-covalent nature of these crosslinkages, they would provide for the viscous component of the rheological behavior of total glutenin. The magnitudes of the viscous and elastic components would depend on the relative amounts of Glu I and Glu II, respectively. Rheological properties of doughs would depend on those of glutenin as modified by other flour constituents.

Doughs from flours of cultivars that contain a greater proportion of Glu II will, therefore, be more elastic and, in turn, have longer development times (during mixing) and greater mixing tolerance. This hypothesis is in general agreement with the correlations obtained by Orth and Bushuk (1972) between the amounts of soluble and insoluble glutenins and the various rheological properties of doughs.

Ultrastructure

When we go beyond the molecular structure, to the level of structure that can be discerned with the scanning electron microscope (Fig. 8), we observe that freeze-dried glutenin of bread wheat forms highly characteristic fibrils (Orth et al, 1973). Durum wheat glutenin and the glutelins of rye and triticale (rye x wheat hybrid) prepared similarly showed distinctly different structures. This microstructure of glutenin, which arises from the molecular structure, determines the functionality of glutenin in gluten and dough, and hence breadmaking capability of wheat flour. However, further work is needed to determine the exact relationship between molecular (particle) structure and ultrastructure as seen in the scanning electron microscope.

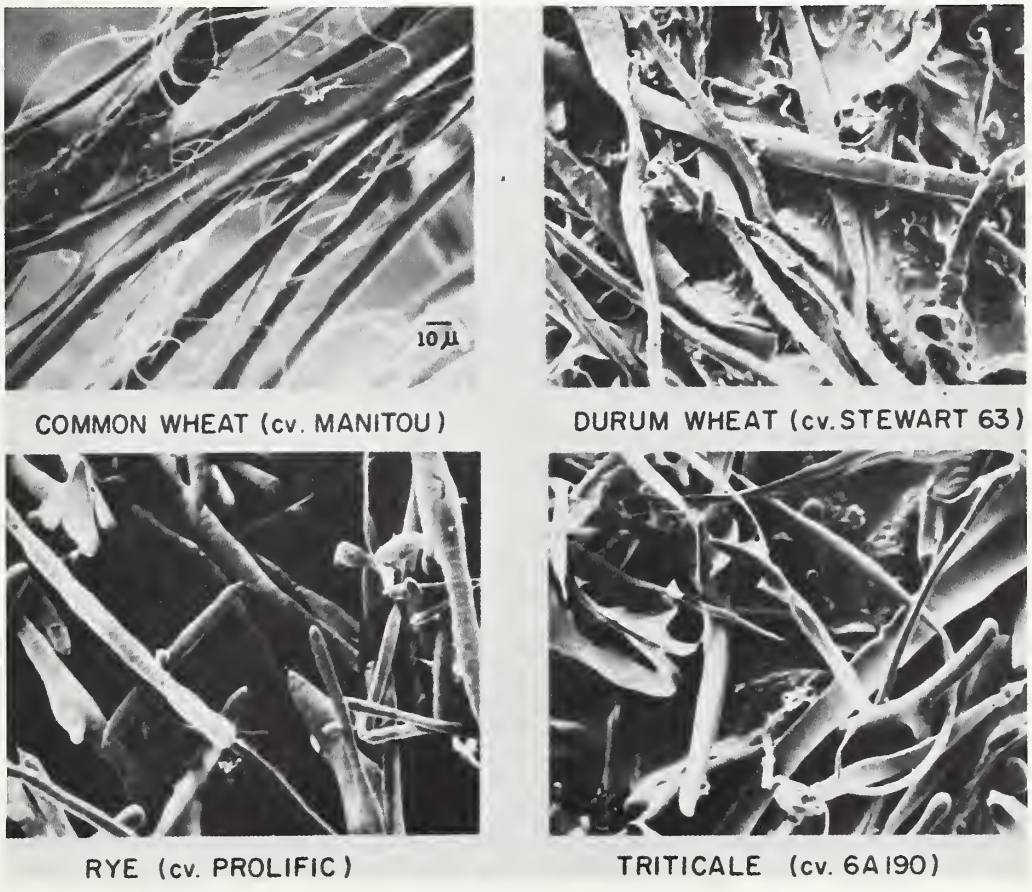

Figure 8. Scanning electron microscope photomicrographs of freeze-dried glutenins of common and durum wheats, and glutelins of rye and triticale.

SUMMARY

Glutenin forms about 35-45% of wheat flour protein. It is important to the breadmaking properties of flour by its contribution of elasticity to dough. It comprises approximately 17 subunits ranging in MW from 12,000 to 134,000. The subunits are held together, in a functional particle, by noncovalent interactions and by disulfide crosslinks. Total glutenin can be fractionated into acetic acid soluble glutenin (Glu I) and acetic acid insoluble glutenin (Glu II). Loaf volume potential of a bread flour depends on the relative quantities of the two types of glutenin. A model for functional glutenin, in which Glu I interacts with other molecules of Glu I and with Glu II molecules through noncovalent linkages, is proposed. Another important property of bread wheat glutenin is its ability to form characteristic fibrils that can be seen in the scanning electron microscope.

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Gluten Protein Interaction with Small Molecules and Ions- the Control of Flour Properties

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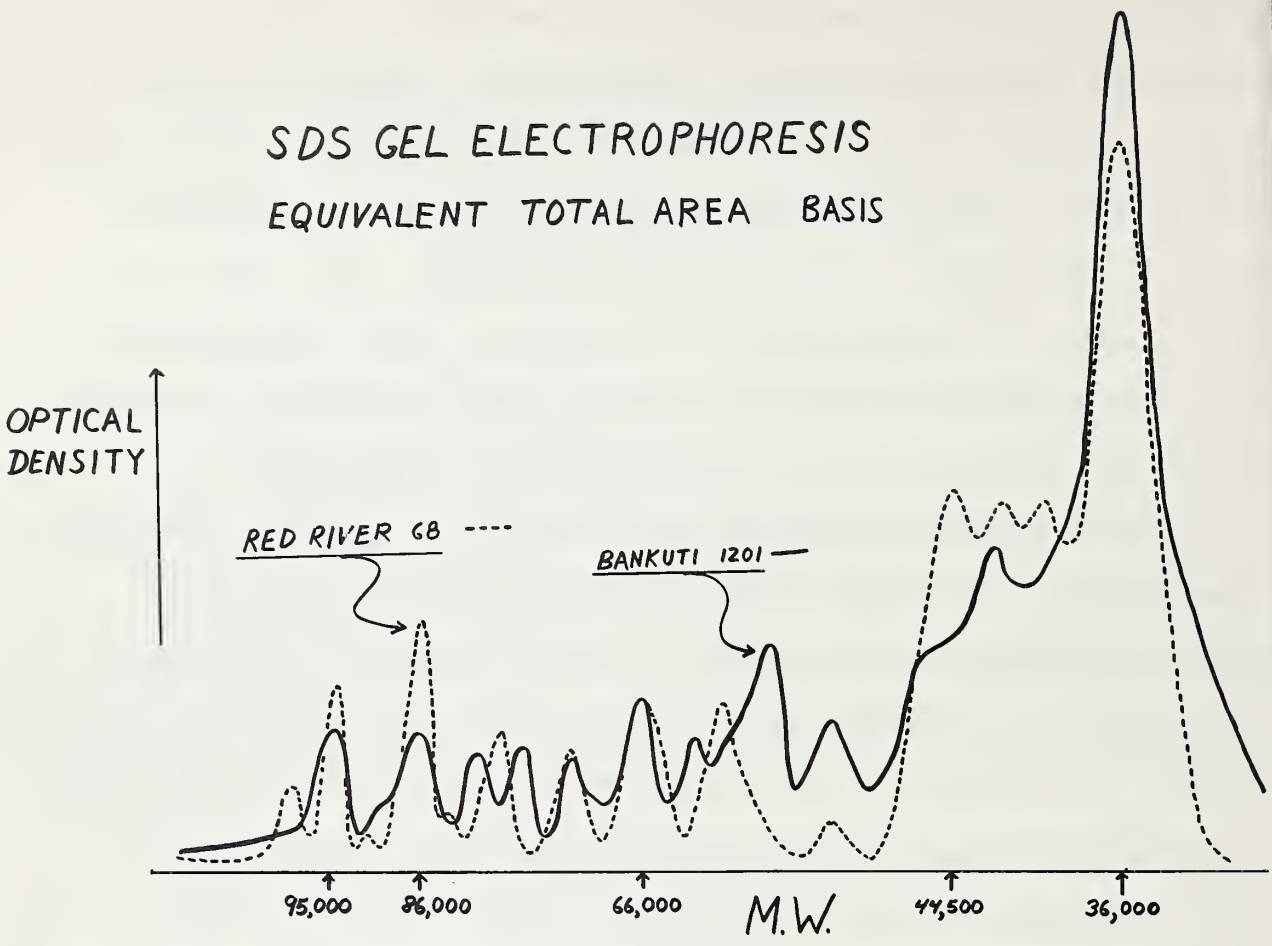
The differences in chemical composition and in physical properties between glutenin and gliadin proteins have been the subject of many reviews. I would like to discuss the similarities of these proteins and show how an understanding of their similar structures provides insight into the changes that occur as flour is mixed into dough and of the effects small ions have on dough properties. When possible I will use the term storage proteins rather than the solubility classes glutenin and gliadin because I want to point out their similarities.

The term storage protein also provides an indication of the role of these proteins in the wheat kernel. They are synthesized and deposited in the developing kernel to be used later, during germination, as a nitrogen and amino acid source. A recognition of this role in the kernel also points out a fact that I must be continually aware of as I conduct research on these proteins, i.e. the cellular machinery that synthesized these proteins as well as the starch, lipid, cell walls, and all sub-cellular organelles in the developing kernel is still present when wheat is milled into flour. The milling operation breaks open the endosperm cells and may also rupture membranes which maintained organization within the cells. This brings into contact molecules which were separate in the developing grain. Mixing a flour-water dough then allows reactions to occur which were not possible in the intact kernel. Add to this flour, sugar, oil,

salt, dough conditioners and improvers, etc. and there are further opportunities for additional reactions. The result is a myriad of reactions. Almost any reaction which is searched for by the cereal chemist can be found. The researcher is then left with the task of sorting out those reactions which are significant in determining dough properties. This is why I want to concentrate on the similarities of the storage proteins and to consider those reactions which are exhibited by 70 to 80% of the protein in the wheat kernel. These are the proteins which will control dough properties.

When the proteins of wheat are extracted from the endosperm with sodium dodecyl sulfate, approximately 98% of the total protein is solubilized. Separation of this protein according to molecular weight on SDSPAGE* shows the majority of the protein falls in the molecular weight range of 35,000 to 45,000. A quantitative estimate of the percentage of protein in this range is obtained by densitometrically scanning the gel and a typical scan is shown for the two varieties Bankuti and Red River 68 in Figure 1 (1). This molecular weight range (35,000 to 45,000) accounts for nearly all the storage proteins (gliadin and glutenin) in wheat and makes up 77% of the total protein in Bankuti and 75% of that in Red River 68. This is the group of proteins whose structure I will discuss. They are compactly folded proteins with a typical globular structure.

*SDSPAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis



The densitometric scans of a sodium dodecylsulfate-polyacrylamide gel electrophoretic separation of SDS wheat endosperm extracts for Bankuti and Red River 68 wheats.

The storage proteins in wheat in addition to having similar molecular weights, also have very similar amino acid compositions. The amino acid in greatest abundance is glutamine and it may constitute one third or more of all amino acids in a single protein (2). If the amino acids are divided into three general classes, non-polar or hydrophobic (e.g. valine, leucine), hydrophilic (e.g. glutamine), and charged (e.g. aspartic acid, arginine), it is clear that the storage proteins have an abundance of hydrophobic and hydrophilic residues and a dearth of charged residues. These classes and their abundance in the storage proteins are summarized in Table 1.

TABLE 1

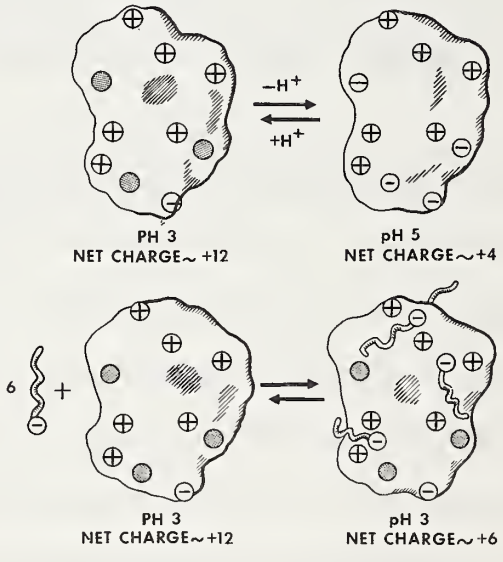
Classes of amino acids in wheat proteins

Percent	Gluten	Glutenin	Gliadin	A-gliadin
Charged residues Lys, His, Arg, Glu, Asp	7	12	8	9
Hydrophobic residues Val, Ile, Leu, Pro, Ala, Phe	39	35	42	38
Hydrophilic residues Gln, Asn	35	28	36	38

The result of this amino acid composition is a family of proteins, compactly folded to minimize hydrophobic residue exposure to the aqueous solution, and with very few charged sites on the surface of the protein. In fact, there are sufficient hydrophobic amino acids that all cannot be buried in the interior of the molecule and some patches of hydrophobic amino acids must be exposed to the solvent at the surface of the protein. This conclusion is also supported by the strong binding of hydrophobic molecules to the storage proteins (3).

The low charge density on the surface of the proteins combined with hydrophobic areas and an abundance of residues capable of forming hydrogen bonds (both hydrophobic interactions and hydrogen bonds could maintain an interaction once formed) result in a high sensitivity to salt concentration. Even a low (e.g. 0.005 M NaCl) salt concentration effectively masks the repulsion of one charged storage protein molecule for another of like charge. This allows a sufficiently close approach of one molecule to another to allow hydrophobic and hydrophilic interactions to form. Therefore, in order to increase or decrease protein-protein interactions, either the charge on the protein must be altered or the salt concentration of the solution must be changed.

The effect of salt concentration on protein aggregation is straightforward and predictable; the effect directly follows ionic strength. Divalent ions will be more effective than monovalent ions, and trivalent ions will be even more effective at reducing the charge-charge repulsion of similarly charged proteins. Much more interesting, however, are specific effects brought about by ions which bind to the protein surface and which bear a charge since this can reduce or increase the net charge on the protein. Particularly interesting are the organic acids and lipid molecules. These compounds strongly bind to the hydrophobic regions on the molecule as is schematically shown in Figure 2. Since the binding constants for this type of interaction are frequently large (3), an effect will be seen at very low ligand concentration.



A schematic representation of wheat gluten storage protein at pH 3 and pH 5 showing the reduction in surface charge that accompanies the ionization of carboxyl side chains in the protein. The lower portion of the figure shows a similar surface charge reduction accompanying the binding of a negatively charged lipid molecule to hydrophobic regions on the protein surface. (Figure courtesy of F.C. Greene).

Using the A-gliadin system which is known to aggregate at pH 5 and 0.005 M KCl, I have studied protein aggregation as influenced by various ions and molecules known to affect dough properties or baking performance. The aggregation of the protein with KCl was used as a reference and all measurements were made at pH 5. Aggregation was measured by the amount of protein that could be sedimented in the ultracentrifuge at 150,000G. Table II shows the compounds tested and whether they were less effective, equal to, or more effective than KCl in promoting aggregation.

TABLE II

AGGREGATION OF A-GLIADIN BY SMALL IONS AT pH 5, KCl REFERENCE

LESS EFFECTIVE	EQUIVALENT	MORE EFFECTIVE
GULONIC ACID	BROMATE	PHYTATE
STEARIC ACID	IODATE	ASCORBIC ACID
CETAB*	MALEATE	FUMARATE
	CYSTEINE	SDS*

*CETAB - cetyltrimethyl ammonium bromide
SDS - sodium dodecyl sulfate

I also estimated the concentration of these compounds in a dough liquor from the amounts normally used in standard recipes. These comparisons are shown in Table III

TABLE III

MOLAR CONCENTRATION FOR EQUIVALENT AGGREGATION		MOLAR CONCENTRATION IN DOUGH LIQUOR*
5×10^{-3}	NaCl	2% = 1.5
5×10^{-4}	Na Fumarate	2000 ppm = 6.2×10^{-2}
4×10^{-4}	Na Phytate	300 ppm = 1.7×10^{-3}
1×10^{-3}	Na Ascorbate	75 ppm = 1.7×10^{-3}

*dough liquor was estimated from the data of Mauritzen and Stewart, Australian Journal of Biological Science, 1965, 18, 173-189. They found a range of 1.8 to 4.1 increase in solution concentration due to moisture absorption by dry flour. An average of the 7 flours studied was taken for this calculation. This average was 2.7.

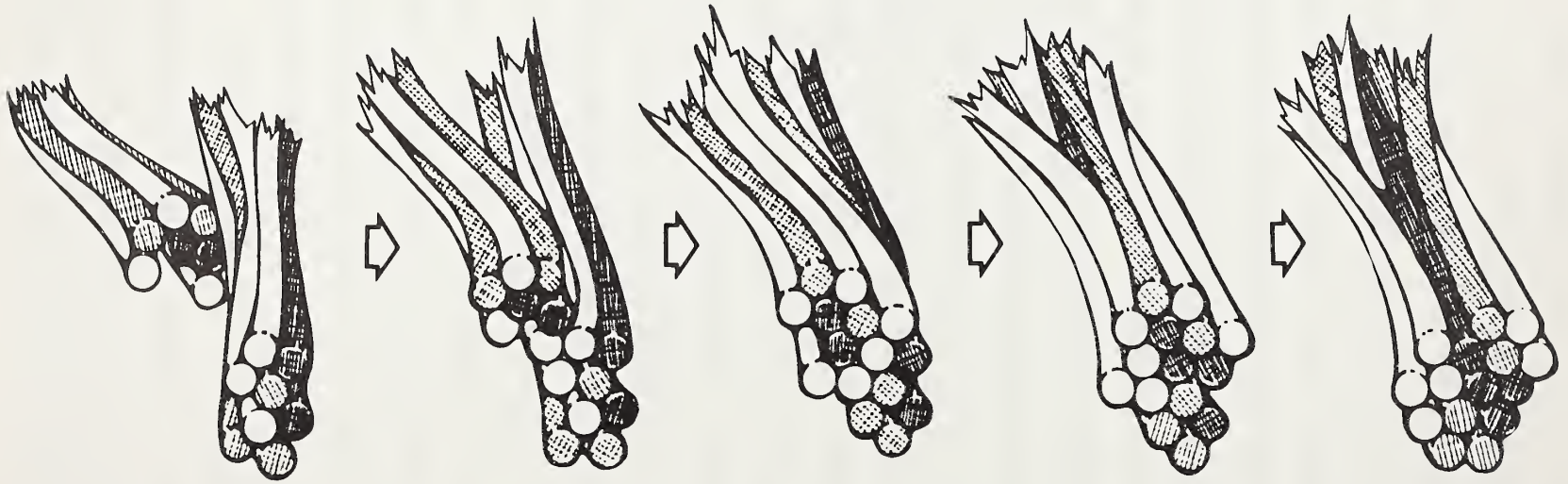
All three compounds that are compared with KCl in Table III, when used in a dough, are clearly present at a sufficient concentration to have a large effect on protein aggregation. In this experiment, only those compounds which have a direct effect on the protein would be effective in this system of purified protein. However, some of these compounds, if added together, might react and their products may affect protein aggregation. Some of the other compounds I tested may have a greater indirect effect on protein aggregation than is shown in this purified protein system, e.g. unsaturated flour lipids may be oxidized by dough improvers to dihydroxy lipids which may bind more strongly to the protein than the original lipid molecule.

While I conducted this study using a fraction of the gliadin proteins, there is good evidence that the other gliadin and glutenin proteins will exhibit similar effects. The A-gliadin fraction has the greatest charge of all the gliadin proteins and therefore will have the greatest charge-charge repulsion. The amino acid composition of all the storage proteins are nearly identical for several of the proteins (4) indicating only small differences exist between the proteins. The glutenin fraction also seems to be very similar to the A-gliadin complex of proteins. Recently, Kobrehel and Bushuk (5) have demonstrated that a glutenin fraction can be solubilized by the addition of stearic acid. This is a good example of a decrease in protein-protein interaction brought about by small ion binding and increasing the charge on the protein. This is also good evidence for the hypothesis that wheat storage proteins interact primarily through secondary (in contrast to covalent disulfide bonds) chemical bonds (6). Earlier, Orth, Dronzek and Bushuk (7), and recently Tu and Tsen (8) demonstrated a quaternary structure for the glutenin proteins similar to the A-gliadin structure (9) and I have

demonstrated this fibrillar structure to be a common structure for the storage proteins in several varieties of wheat (6).

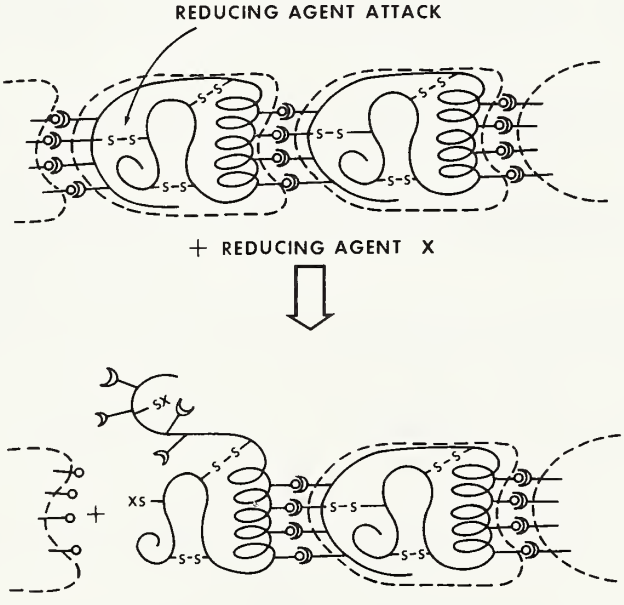
While the glutenin and gliadin molecules apparently interact in a similar manner, all interactions do not necessarily have to be equally strong. Variations in protein-protein interaction will result in the continual exchange of interacting molecules until a set of interactions are formed which maximize interfibrillar interaction energy. Consider a number of flour particles in an aqueous solution. When wet, the protein fibrils spread into the surrounding solution (10) and begin to interact with fibrils from adjacent flour particles. If we group the fibrils into three classes depending on their interaction potential, a sequence such as that shown in Figure 3 will follow. The initial interactions may involve any of the three classes. However, as mixing begins, the fibrils will slide along one another, be pulled apart, and eventually collide again or otherwise interact with another fibril. This process will continue until those fibrils which interact most strongly with one another are more often in contact. An optimally mixed dough would probably correspond to the third position of the sequence shown in Figure 3. An overmixed dough would correspond to the far right position where there would be clumps of protein which contained strongly interacting proteins in the center and weakly interacting protein on the outside. These weakly interacting proteins would be unable to maintain the strongly interacting three dimensional structure of storage proteins characteristic of a well developed dough.

The amino acid compositions of the storage proteins present in most seeds are very similar. Therefore, the unique viscoelastic properties of the wheat storage proteins must reside in their structure and in the structures



The changes in fibril-fibril interaction that occur as mixing proceeds are schematically represented by the three shadings of the fibrils in the figure. Strongly interacting fibrils (dark shading) tend to retain interactions eventually resulting in a strongly interacting core surrounded by more weakly interacting fibrils (clear shading). An optimally mixed dough would correspond to the center of the figure where the average interaction between all fibrils in a dough would be a maximum.

formed as the molecules aggregate. Rheological data (11a,b) has indicated that the interacting network of storage protein in a dough is not covalently bonded yet the disulfide theory has persisted largely because there has been no adequate alternative and because the effect of disulfide reagents on dough properties is so dramatic. The effect of disulfide bond breakage on a noncovalently interacting protein system could also be dramatic since it could disrupt the molecular structure necessary for the formation of the fibrillar structure. A schematic representation of this effect is shown Figure 4. Cysteine was tested for its ability to aggregate the protein as



The effect of breaking an intramolecular disulfide bond on the stability of a fibril. The incorporation of a charged residue into the interior of a protein molecule could alter the conformation of the molecule sufficiently to disrupt the fibril and have a pronounced effect on dough properties.

indicated in Table II. The amount of cysteine used in this experiment would result in complete reduction of the disulfide bonds in the protein and, even

though a mixed disulfide would be formed as an intermediate in the reduction reaction, there would not be a retention of the charged cysteine in the interior of the protein - there would only be SH. Limited amounts of cysteine or similar reducing agents would produce the effect noted in Figure 4.

More work obviously needs to be done on the effects of various dough improvers and "dough active" agents. The mechanism of many of these improvers may be different from the currently proposed mechanisms.

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INFLUENCE OF COMPOSITION ON WHEAT FLOUR DOUGH PERFORMANCE

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It is said that we currently live in the "Age of Polymers." Most biological systems are based on polymers, including the food we eat. Natural polymers such as cellulose, resins, gums, and asphalt have been used by man for thousands of years. However, today many of the materials we use in daily life--plastics like polyethylene, fibers such as nylon and dacron, and artificial rubber--are man-made synthetic polymers. The widespread use of polymers has led to increased study of such materials, provided man tools for their characterization, and given us greater insight into their properties. The unique cohesive elastic properties of wheat flour doughs are due to their constituent polymer components: proteins, pentosans, and starch. In this paper I shall try to show how variations in wheat proteins influence the behavior of dough and how it is possible by introducing other polymers such as gums or bacterial polysaccharides (PS) to alter the physical behavior of doughs from flours varying in baking quality.

A polymer is described as a large molecule built up of small molecules either of identical repeating units or a number of different units. Proteins are generally formed by a varying combination of 18 different amino acids. The different number of proteins that can be formed is probably infinite.

Polymer solutions are thick and have high viscosities because of the high molecular weights and interactions of the molecules. The molecules tend to associate because of hydrogen bonding and hydrophobic bonding of groups on their surfaces.

The mixing of dough involves primarily the alignment and interaction of the protein molecules to form a continuous matrix in which starch and CO_2 are held. Figure 1 compares the results of mixing strong, medium, and weak wheat flour doughs (1,2). Resistance of the dough is shown on the vertical axis and time on the horizontal axis. The excessively strong wheat (Red River 68) requires a longer mixing time to achieve the maximum resistance and maintains its viscosity longer than the good wheat. In contrast, the weak wheat rapidly achieves maximum resistance but retains it for only a short time. In our research, we have been seeking to identify the nature of the protein variations resulting in differences in dough behavior between wheat flours differing in baking quality.

Differences in Proteins of Wheats Varying in Baking Quality

Difficulties in studying polymers are due to their high molecular weight (MW) and often to poor solubility which makes them difficult to isolate and characterize. The wheat proteins certainly fit that description. During the past 20 years, new tools have become available which greatly

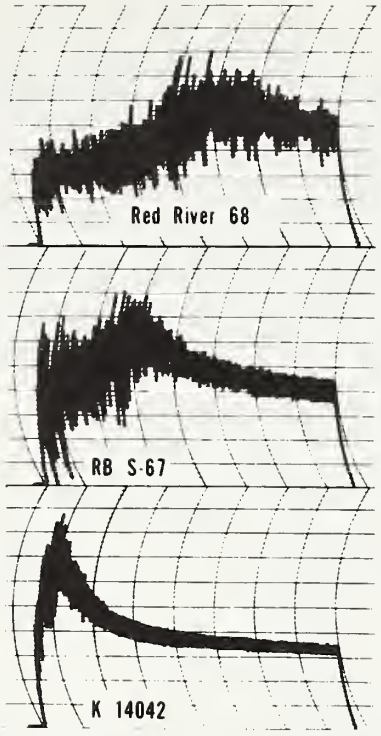

Figure 1. Mixograms of very strong, medium, and weak wheats.

facilitate methods of separating proteins. Gel permeation chromatography is one of those methods. Figure 2 diagrams the principle of operation

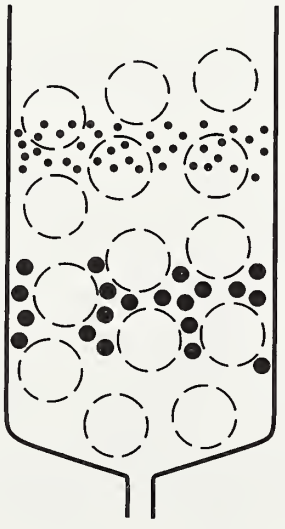

Figure 2. Diagram of a gel permeation column.

of gel filtration. The protein, dissolved in an aqueous solvent which dissociates the molecules, is passed through a column composed of porous beads. The large molecules pass around the porous beads, since the openings are too small for them to enter and, therefore, they elute from the column first. The small molecules pass in and out of the porous beads and are thereby retarded and eluted later. This technique has been an important method for the study of wheat proteins and much of our work depends on it.

In Figure 3 are shown the results of applying fractions of wheat

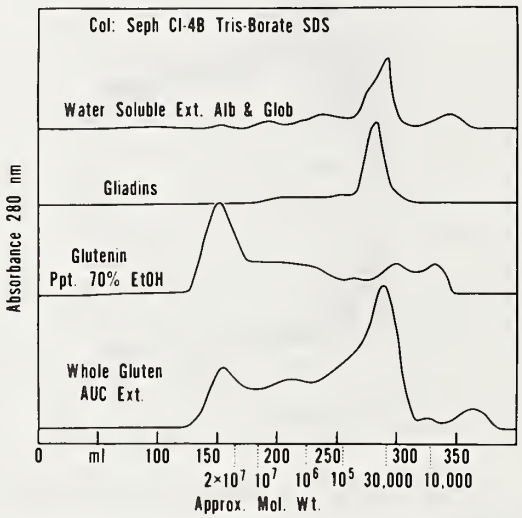

Figure 3. Sepharose Cl-4B filtration of wheat protein fractions.

proteins, prepared by extracting the flour with different solvents, to an agarose gel permeation column. The buffer contains sodium dodecyl sulfate, which prevents hydrophobic bonding between the proteins; whereas most previous work was with urea or guanidine hydrochloride which prevented hydrogen bonding. The vertical axis is the absorbance at 280 nm, which indicates the concentration of the protein in the effluent. The horizontal axis indicates the volume of effluent required to elute the protein from the column and the approximate MW of the protein as measured by the elution volume. The top diagram is the result obtained by a water extract of a Ponca wheat flour plus some added gliadin. Most of the main peak is composed of low MW albumins and globulins plus the small amount of gliadins and trace amounts of some higher MW proteins. The second diagram is the elution pattern of gliadin that was isolated by dissolving the gluten in dilute acetic acid and then precipitating glutenin from a 70% ethanol solution made neutral with NaOH. The 70% EtOH soluble gliadin fraction was then applied to a Sephadex G-100 column and the high MW gliadin and omega gliadins were separated from the remaining gliadins, which are all approximately 35,000 MW in this sample.

The third elution profile is that of the glutenin that was separated from the gliadin above by precipitation (twice) from neutral 70% EtOH. In addition to early eluting high MW protein, which could be as high as 15-20 million daltons, there is a second broad peak containing protein

with a wide range of MW's. Unlike most other gel permeation columns, this agarose column separates glutenin proteins of very high MW's. Next there are two small peaks with apparent MW's below that of the gliadins and the water solubles. In the last elution profile are recorded the results of the whole gluten extracted with a buffer containing acetic acid, urea, and cetyltrimethylammonium bromide (AUC) (3) which extracts more of the glutenin than just acetic acid alone. The two small peaks at about 340 and 360 ml in the top and bottom profiles are very low MW peptides, sugars, and salts. Thus there is a spectrum of proteins in wheat that differ in molecular weight and amino acid composition.

Recently Huebner and Wall (4) extracted the proteins from wheat flours of different baking quality with the AUC buffer. The proteins were then separated into different MW fractions on an agarose column in 5.5 M guanidine HCl (Fig. 4). The first peak that is eluted at the

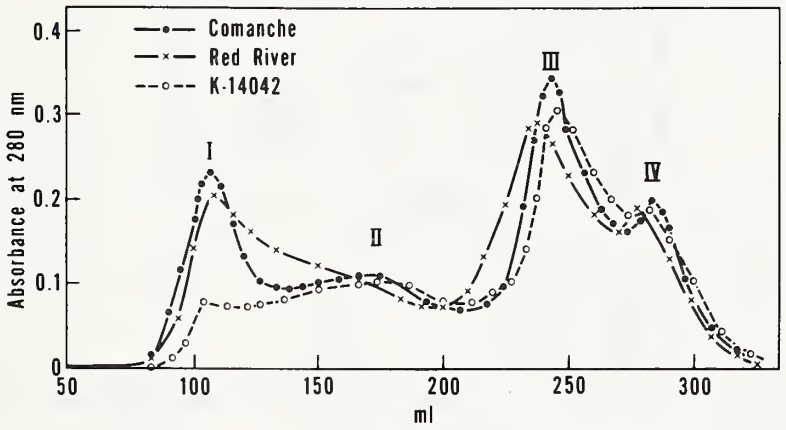

Figure 4. Gel filtration of AUC extracts from three wheat flours on Sepharose 4B. Solvent: 5.5 M guanidine HCl.

void volume could have an apparent MW of over 20 million. The strongest (Red River 68) and weakest (K-14042) wheats used here were the same as used for Figure 1, whereas the medium strength wheats were different varieties but similar in physical characteristics. It was found that protein extracts from excessively strong wheats contained a large quantity of the first eluting peak and the weak wheats a very small amount of that high MW protein. The good quality wheats contained amounts intermediate between the two extremes. While the amounts of gliadins, albumins, and globulins did not appear to vary among varieties, there was a general increase in these proteins when the Glut-I peak decreased.

In addition to the two glutenin fractions found in peaks I and II (Fig. 4), there is also some unextracted protein left called the insoluble residue protein. When the percentage of the residue protein was high in a wheat flour, the flour dough was also long mixing. While a number of people have worked on this problem, Orth and Bushuk (5) were the first to suggest that the residue protein was positively related to the mixing and baking quality of the wheat and that the acetic acid-extracted

glutenin negatively correlated. In this study (4) a number of wheats of varying qualities were analyzed by this method and a definite relationship was found between the percentages of the various fractions and the mixing and baking quality of that wheat. The higher MW proteins contributed to longer mixing times and a better loaf of bread, but only to a certain degree. If there was too much of the high MW protein, the mixing time was too long and the loaf volume was also lower. Evidently the protein was too tough or cohesive, preventing the dough from expanding optimally during the fermentation and baking.

Figure 5 illustrates the optimum percentages of the various protein

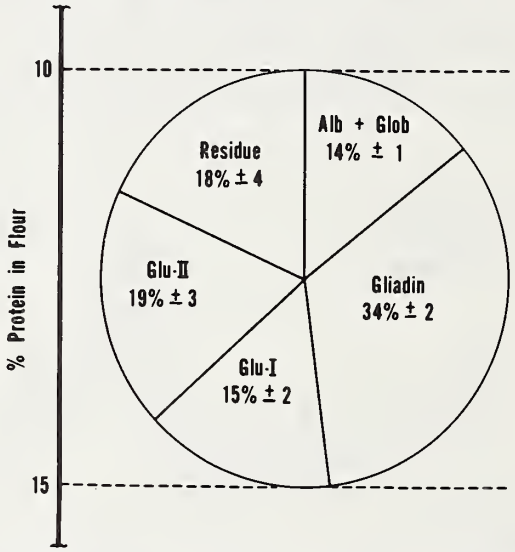

Figure 5. Optimum percentages of wheat proteins required for a good bread-baking wheat.

fractions found in the good bread-baking-quality wheats. Not only is the right ratio of the fractions important but also the total flour protein, which was found to lie between 10-15%. It is possible that a wheat with amounts or ratios of proteins outside those shown here could also give a suitable bread, especially if some additives are used. Orth has now suggested that the quantity of residue protein is correlated to the mixing characteristics of wheat and could be used to identify good baking wheats (6).

Disulfide Cleavage

The high MW of native glutenin is at least partly due to the presence of disulfides linking smaller proteins together. To study these smaller proteins, the glutenin disulfides have been cleaved. In Figure 6, a diagram is given to demonstrate how the disulfide bonds of cysteine are broken and the resulting sulfhydyl groups then alkylated to prevent the disulfides from being formed again. This has made it possible to then separate the constituent proteins on gel filtration columns and thereby determine their physical and chemical characteristics.

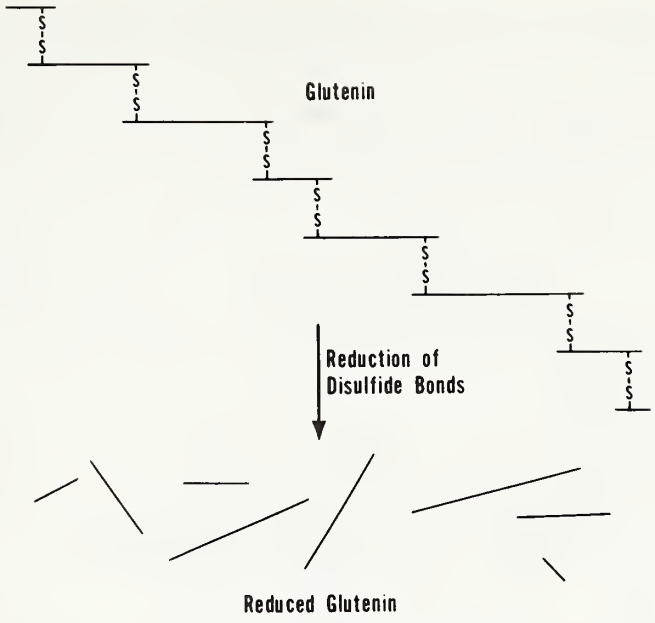

Figure 6. Cleavage of disulfide bonds in glutenin.

As shown in Figure 7, Huebner and Wall (7) separated the reduced

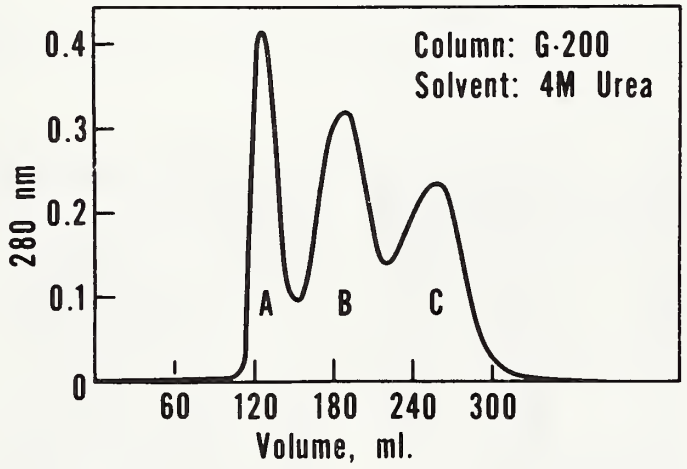

Figure 7. Gel filtration of reduced glutenins from Ponca Wheat (Ref. 7).

glutenins into three MW fractions on Sephadex G-200 columns in 4 M urea. The A fraction which elutes first should be the highest MW fraction; however, it was found to consist of a number of lower MW proteins upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE). Recent studies suggest that this fraction may consist of smaller MW membrane proteins that are highly aggregated through hydrophobic groups and are not actually part of the disulfide crosslinked glutenin fraction. The B fraction contains the main long-chain polypeptides usually seen upon starch gel electrophoresis (8) and on SDS PAGE (9) as the slow

moving bands. The MW's are in the range of 80-130,000. The C fraction contains polypeptides in the MW range of 40-45,000, similar to the gliadins.

The B fraction was also subjected to ion exchange chromatography (7), and some individual components were isolated as shown in Figure 8.

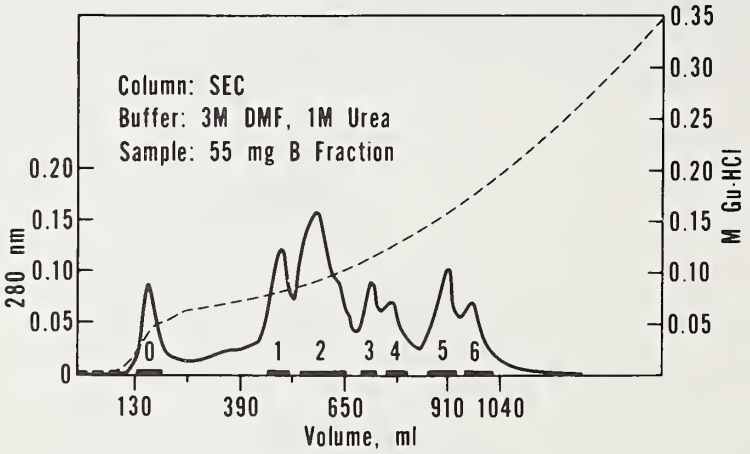

Figure 8. Chromatographic separation of B fraction from Figure 7 on sulfoethyl cellulose (Ref. 7).

The C fraction upon ion exchange chromatography gave only limited separation. This fraction of proteins appeared to be very complex which prevented resolution of individual components.

The amino acid analysis of these various fractions provided definite proof that glutenin is considerably different than the gliadins (10). Earlier amino acid studies of the whole glutenin failed to show any large differences, since the three fractions together masked each other and gave an average amino acid content that appeared similar to the gliadins. Figure 9 illustrates some of the major differences among

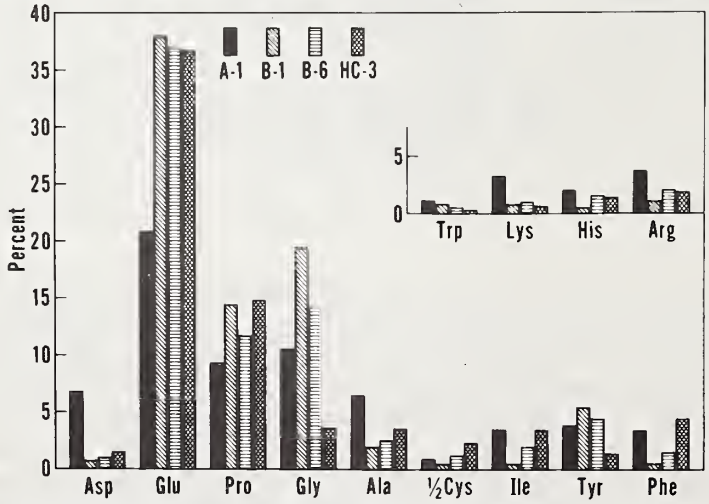

Figure 9. Percentages of some amino acids from four fractions A-1, B-1, B-6, and HC-3 taken from Ref. 10, Table 1.

amino acids in these three fractions. The A fraction contains much more of the basic amino acids and less glutamine and proline and is somewhat similar to the water solubles. The B fraction contains high amounts of glutamine, proline, and glycine with up to 72% of the protein consisting of these three amino acids. The composition is therefore similar to the structural proteins, such as collagen, that yield long fibers. The C fraction is very similar to the gliadins except for more cysteine. This would lend itself to more inter-and intra-molecular disulfide bonding and disulfide interchange during dough mixing.

Recently, Arakawa et al. (11) also separated the reduced glutenins into three fractions from a number of wheat varieties varying in quality, by this procedure. They found that the ratio of the three fractions varied among the varieties, but they made no attempt to relate the variation to mixing or baking qualities. They compared the aggregating characteristics of each of the fractions and of the whole glutenin and gluten. They found that the A and B fractions aggregated into larger particles by themselves than for the whole glutenin. The aggregation curves were similar for each of the wheats within each of the three fractions (A, B, and C). There were slight variations, however, indicating minor variations in the components from each variety. The greatest similarities were found among the C fractions. This work adds more evidence to the hypothesis that the differences among the mixing and baking qualities of the wheats are due to the differences found in the glutenin fraction.

Rheology of Doughs

In Figure 10 is shown a picture from the scanning electron microscope

Figure 10. Scanning electron microscopy of wheat flour dough.

of wheat flour dough after mixing. We can see how the wheat gluten proteins envelop the starch granules to form the dough matrix. Without the proper ratio of protein polymers, the protein film covering the starch granules would not hold together properly. In the case of the soft wheats, there are too few of the protein molecules to make a good loaf of bread. Perhaps the addition of some other polymers would eliminate this deficiency.

Polysaccharides

Polysaccharides (PS) are also polymers and because of their characteristics, such as very high viscosities and hydrogen bonding interactions with proteins, they might be useful in the baking of bread. At the Northern Research Center, numerous extracellular PS have been produced by either yeasts or bacteria. Each of these have different characteristics and, because of the previous experience with xanthan gum and dough mixing (12), a number of these PS were tested with a standard baking soft wheat flour.

In Figure 11a is illustrated the curve formed by mixing a standard

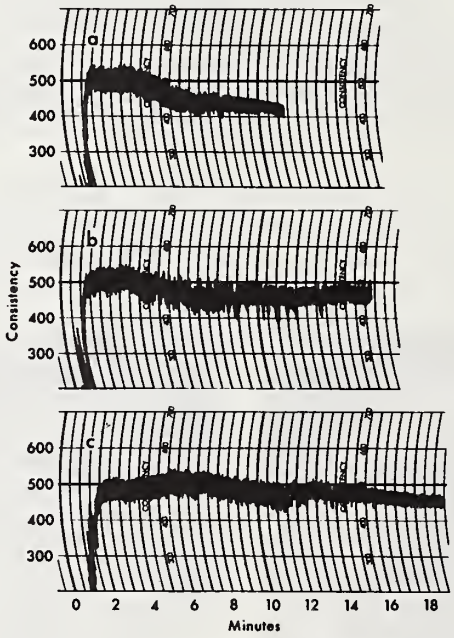

Figure 11. Farinograph curves of 50 g standard mix SRW wheat flours. (a) No additives, 27.5 ml H_2O. (b) Added 0.3 g PS B-1459, 29.5 ml H_2O. (c) Added 0.075 g calcium carrageenan, 27.8 ml H_2O.

SRW wheat in a farinograph. In Figure 11b and 11c the farinograph curves increase in mixing time and stability following addition of as little as 0.3% PS B-1459 and 0.15% of calcium carrageenan, respectively. In Figure 12, we see the results of adding 0.1% PS B-1973 in three

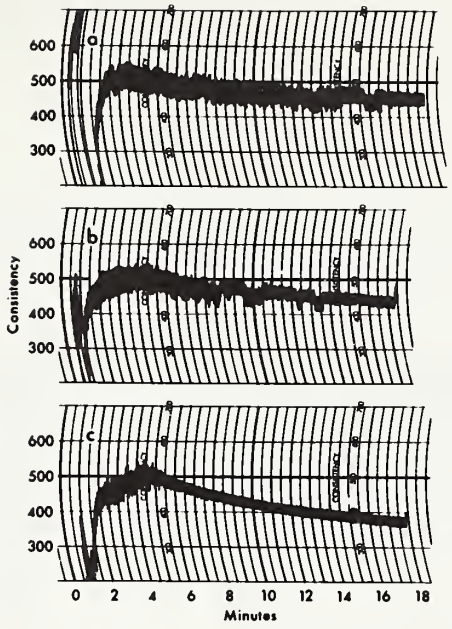

Figure 12. Farinograph curves of 50 g standard mix SRW wheat flours. (a) Added 0.15 g PS B-1973, 28.7 ml H_2O. (b) Added 0.1 g PS B-1973 (old sample), 28.8 ml H_2O. (c) Added 0.1 g PS B-1973 (deacetylated), 28.2 ml H_2O.

different forms. In Figure 12a the PS is fully acetylated with 25% of the PS being acetyl groups. In 12b, it has lost some of the acetyl groups and in 12c, it has been completely deacetylated. While only two of the PS tested actually increased the mixing and stability times significantly, other characteristics were found that may be of interest.

Along with these PS's, Na alginate and three carrageenans were also tested. They were found to be even more effective in increasing the mixing times. Since the carrageenans gave two peaks, one at about 1.5 min and the second at 8-12 min, the amount was kept very low, and a small amount of a specific PS was also added which eliminated the two peaks. By adding specific amounts of carrageenans and PS, nearly any type of mixing curve could be obtained on the farinograph. As of now, no baking tests have been made so it is not known if they would also improve the loaf volume and structure.

In addition, crude gluten extracted from the wheat was reacted with PS in solution at various concentrations to determine the interactions of proteins and the PS. Each PS was found to be different in its effect on gluten solutions, as seen in Figures 13a and 13b. Some formed no turbidity at all, whereas some interacted very strongly with the protein in solution. The results indicated that a certain ratio of the two components was necessary for the interaction to take place and that each reaction was different. Two PS's, B-1459 and B-1973, reacted very strongly to form stringy precipitates at very low concentrations, as low as 0.002%.

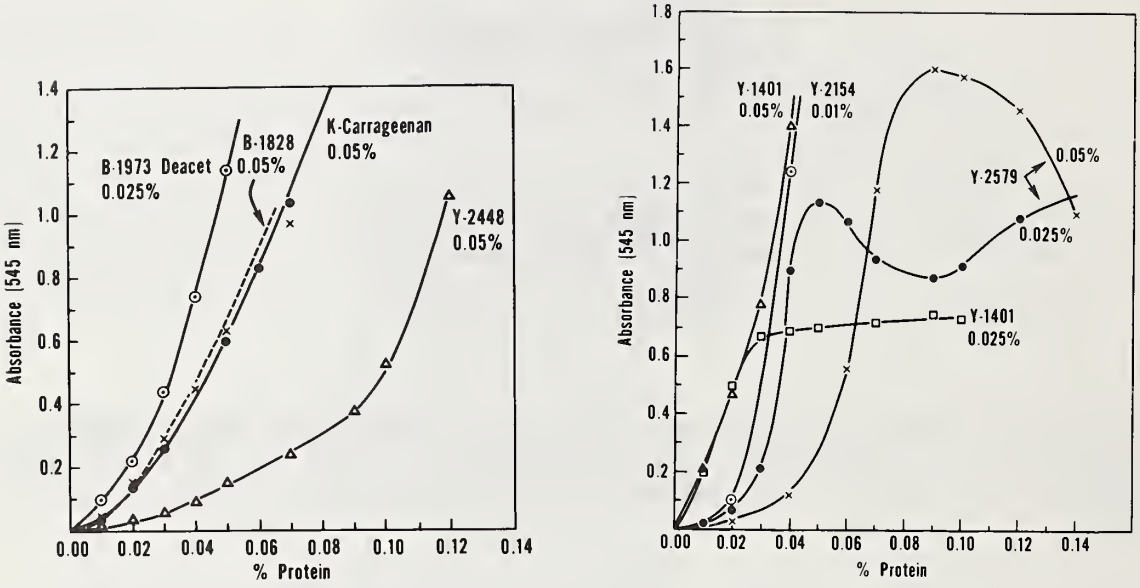

Figure 13. (a,b) SRW wheat gluten-polysaccharide turbidity curves in 0.01 M acetic acid. Percentage of polysaccharides in final solution given next to each curve, percentage protein at base of graph.

These experiments provide further evidence that the properties of wheat gluten proteins can be modified by the presence of PS. Often very high-yielding wheats are lower in protein and baking quality than our good bread wheats as well as some high protein wheats which produce very poor bread. Perhaps we will have to introduce low cost additives such as PS which will permit use of such wheats in breads if we need to extend our supply of bread wheat.

Conclusion

We have shown that the rheological properties of wheat doughs can vary with their constituent proteins. Selection of wheats for optimum protein content and quality as indicated by amount of high MW glutenin and residue protein could be a useful tool in wheat breeding. Alternatively, low-cost additives that facilitate interaction among wheat proteins may

be a means of utilizing more of the poorer baking quality wheats for the production of bread.

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INCREASED WHEAT EXTRACTION RATES

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ABSTRACT

Eighty percent of the taste panel members found no difference in mastication or in taste in comparing bread produced from straight-grade flour and extended extraction flour (Table I). Further studies were continued on the production of extended extraction flour using a bran finisher and a combination of a bran finisher (BF) with a pin mill (PM)

TABLE I

TASTE PANEL RESULTS ON STRAIGHT-GRADE VERSUS EXTENDED EXTRACTION FLOUR

Number of Members that Rated the Extended Extraction Flour Bread as:				
SAME	SLIGHTLY TOUGH	SLIGHTLY GUMMY	SLIGHTLY BLAND	SLIGHTLY BITTER
6	1	1	1	1

Compared with the Straight-Grade Flour.

80% found no difference in mastication.

80% found no difference in taste.

and Entoloter (ENT). Two hard red spring wheat cultivars (Era and Waldron) were used in the study. Comparison of the data between the straight-grade and extended extraction flour for Era (Table II) and Waldron (Table III) indicated that the best combination was to use the Entoleter on the bran and head shorts, and the bran finisher on the tail stock from the reduction side of the mill. The cumulative ash curve for the Waldron (Figure 1), graphically illustrated the differences between the three milling procedures.

TABLE II

COMPARISON OF EXTENDED EXTRACTION FLOURS FROM THE HARD RED SPRING WHEAT VARIETY WALDRON PRODUCED BY THREE MILLING PROCESSES

FLOUR	STRAIGHT-GRADE	EXTENDED[a/]		
	%	BF %	ENT + PM %	BF + ENT + PM %
Extraction (%)	74.97	80.34	78.77	79.46
Ash (%) b/	0.41	0.64	0.57	0.58
Protein (%) b/	14.10	15.10	14.50	14.50
Agtron Value	63.00	40.50	47.00	45.50

TABLE III

COMPARISON OF EXTENDED EXTRACTION FLOURS FROM THE HARD RED SPRING WHEAT VARIETY ERA PRODUCED BY TWO MILLING PROCESSES

FLOUR	STRAIGHT-GRADE	EXTENDED[a/]		
	%	BF %	ENT + PM %	BF + ENT + PM %
Extraction (%)	77.44	-	80.89	80.87
Ash (%) b/	0.46	-	0.62	0.58
Protein (%) b/	12.70	-	13.10	13.00
Agtron VAlue	64.50	-	46.00	48.00

a/ BF = bran finisher used on all offals; ENT + PM = bran and head shorts 1 pass thru entoleter and 1 pass thru pin mill plus tail stock thru pin mill twice; BF + ENT + PM = tail stock thru bran finisher twice and bran and head shorts thru entoleter then thru pin mill.

b/ 14% moisture basis.

The baking data (Table IV), showed slightly poorer crumb color for the combination series of BF + ENT + PM, but higher bake absorption than the straight-grade flour. As indicated from previous taste panels, it would be difficult to distinguish between the two flours. Also, with the bran finishers incorporated into the mill flow, it appears a shorter milling system could be employed.

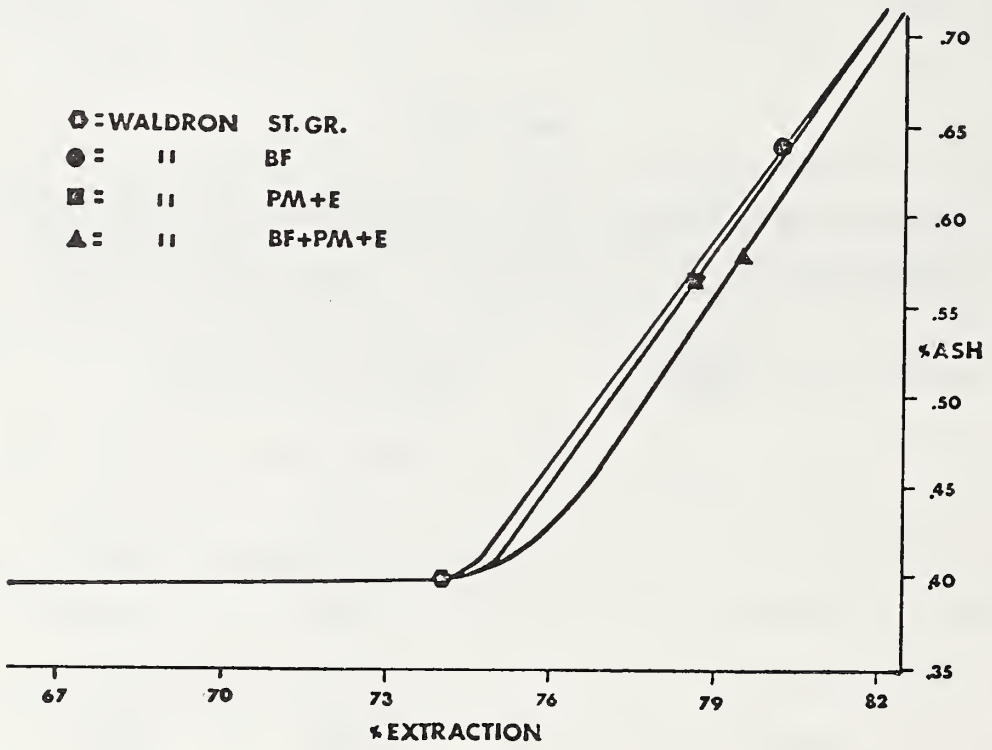

Figure 1.--Cumulative ash curve for Waldron. ST.GR. = Straight-grade flour; BF=bran finisher used on all offals; ENT + PM = bran and head shorts 1 pass thru entoleter and 1 pass thru pin mill plus tail stock thru pin mill twice; BF + ENT + PM = tail stock thru bran finisher twice and bran and head shorts thru entoleter then thru pin mill.

TABLE IV

FLOUR AND BAKING CHARACTERIZATION OF EXTENDED EXTRACTION FLOUR FOR ERA AND WALDRON HARD RED SPRING CULTIVARS PRODUCED BY DIFFERENT MILLING PROCESSES

VARIETY	FLOUR[a/]	MIXOGRAM PATTERN[b/]	BAKE ABSORPTION[c/] %	MIXING TIME min	CRUMB COLOR	LOAF VOLUME cc
ERA	SG	3	59.8	3.00	101	940
"	ENT + PM	3	59.6	3.00	98	953
"	BF + ENT + PM	3	60.3	3.00	98	933
WALDRON	SG	4	61.5	3.25	99	978
"	ENT + PM	3	62.3	3.00	98	965
"	BF + ENT + PM	4	62.6	3.00	97	968
"	BF	4	62.7	3.00	94	955

a/ SG = straight-grade flour; BF = bran finisher used on all offals; ENT + PM = bran and head shorts 1 pass thru entoleter and once thru pin mill plus tail stock thru pin mill twice; BF + ENT + PM = tail stock thru bran finsher twice and bran and shorts thru entoleter then thru pin mill.

b/ 1 = weak to 10 = very, very strong.

c/ 14% moisture basis.

EFFECTS OF SPROUTING ON NUTRITIONAL VALUE OF WHEAT

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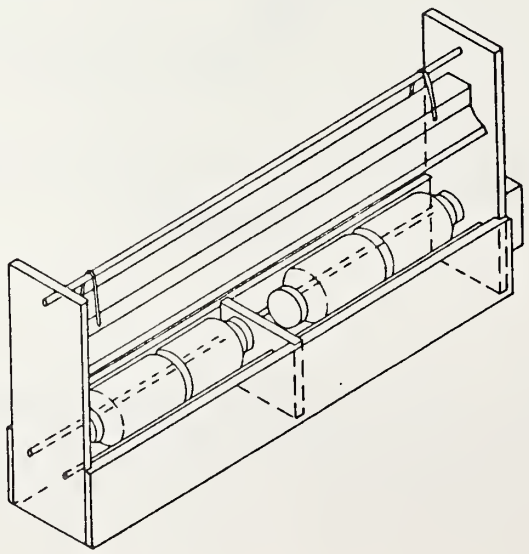
Wheat has historically been considered the staff of life. Rightly so, because it has many properties that make it the most widely consumed food in the world. No food is perfect, wheat being no exception; lysine being the first limiting amino acid in a wheat diet.

Pomeranz (1974) reported barley malt sprouts contain two and one-half times as much protein as barley and that the sprout proteins are almost twice as rich in lysine as the barley proteins. If sprouted barley sprouts contain more protein and more lysine, wheat could have a similar increase in protein and lysine content.

Work was initiated to study the effects of sprouting on the protein, lysine, vitamin A and ascorbic acid content of wheat sprouts. Work was also planned to study the potential acceptance of wheat sprouts as a dietary item to determine whether the consumer would purchase the sprouts and use them.

Initial work was conducted with Hard Red Winter wheat. However, representative cultivars of each class of wheat were sprouted and analyzed for nutritive content. Development of sprouting procedures to produce a mold-free, wholesome product was the first challenge. The routine procedures for sprouting alfalfa and mung beans were found to be unsatisfactory for sprouting wheat. A device was developed (Figure 1) to gently agitate the seeds every four hours in addition to daily rinsing. The use of this procedure resulted in the most satisfactory results.

Figure 1. Diagram of a device for agitating wheat sprouts for the production of mold-free sprouts.



The use of chlorinated water did not prove to be helpful in controlling mold when the sprouts were turned every four hours and rinsed daily. The use of chlorinated water only added to the cost factor and would have raised the problem of the use of chemicals being objectionable to some potential customers.

After a satisfactory sprouting procedure was developed, all samples were subjected to the following procedure. A 500 gm sample of wheat was placed in a one gallon jar. The wheat was rinsed with fresh tap water, which was drained off after five minutes. Two hundred ml of fresh tap water was then added to the wheat sample. The jar was placed on the sprouting device. Each jar was equipped with a screened lid to permit aeration of the sample. The sample was thoroughly rinsed and shaken daily to control mold growth and keep the sprouts separated from each other. Some were sprouted under continuous fluorescent light and others were sprouted in the dark to determine the effects of light on nutritive quality. Daily, a portion of the sprouts was withdrawn, dried in a convection oven at 43°C., and ground for amino acid analysis. Fresh samples were withdrawn and used for proximate and mineral analyses and for vitamin A and ascorbic acid content.

Wheat sprouts were bagged in 4 oz quantities in small polyethylene bags with a single fold label for test marketing in local groceries. The label contained suggestions for use as well as weight and other essential information. No advertising was conducted. Some of these packages were used for shelf life studies by storing at both room temperature and in the refrigerator. The store displays were checked frequently for condition and appearance of the product.

A critical part of this consumer study was considered to be the need for information on how to use the sprouts. Effort was initiated to develop recipes to overcome this problem. A number of interested students became involved, using wheat sprouts in such dishes as salads, soups, entrees, sandwiches, desserts, candies, snacks and oriental foods. One student conducted a taste panel on egg rolls in which wheat sprouts had been substituted for a portion of the fresh vegetables.

Sprouting for seven days increased the protein content approximately 10%, whether it was a low protein wheat at 10% or a high protein wheat at 18% (Table 1). Light had no effect on protein content.

Table 1. Effects of sprouting on protein content of five classes of wheat.

Class	Days of Sprouting		
	0	3	7
Hard Red Spring	17.8	18.1	18.7
Soft White	11.6	12.8	13.8
Soft Red Winter	12.4	13.3	13.3
Durum	14.5	14.5	15.2
Hard Red Winter	9.8	10.9	11.6

The lysine content (Table 2) was increased 15-27% protein, with the greater increase in the low protein wheat and the least increase in the high protein wheat.

Table 2. Representative amino acid analysis of wheat as affected by the sprouting process. (Data based on "as received" moisture content.)

	Hard Red Winter			Hard Red Summer		
	Days of Sprouting			Days of Sprouting		
	0	3	7	0	3	7
Alanine	0.38	0.45	0.43	0.53	0.57	0.58
Valine	0.40	0.27	0.30	0.60	0.44	0.52
Glycine	0.42	0.40	0.45	0.63	0.60	0.67
Isoleucine	0.30	0.19	0.31	0.48	0.33	0.40
Leucine	0.66	0.58	0.68	1.03	0.95	1.05
Proline	0.93	0.84	1.03	1.69	1.75	1.88
Threonine	0.31	0.26	0.33	0.44	0.40	0.47
Serine	0.51	0.50	0.51	0.76	0.85	0.85
Methionine	0.14	0.11	0.15	0.20	0.23	0.20
Hydroxyproline	--	--	--	--	--	--
Phenylalanine	0.44	0.39	0.48	0.78	0.74	0.82
Aspartic Acid	0.52	0.55	0.74	0.77	0.75	0.94
Glutamic Acid	2.68	2.44	2.55	4.88	4.73	4.96
Tyrosine	0.31	0.32	0.32	0.43	0.50	0.51
Lysine	0.30	0.31	0.38	0.39	0.39	0.45
Histidine	0.31	0.28	0.25	0.37	0.41	0.38
Arginine	0.46	0.43	0.54	0.61	0.67	0.73
Cystine/2	0.16	0.15	0.19	0.25	0.26	0.28
Tryptophan						
Total	9.23	8.47	9.64	14.84	14.57	15.69

Ascorbic acid content of the wheat sprouts was found to be minimal at 8 mg/lb of fresh sprouts. Vitamin A content, as carotene, was found to vary from 100-400 mg/lb in the grain and from 400-600 mg/lb in the seven day sprouts (Table 3). Those grains low in carotene intitially increased the most while dry seeds, high in carotene, increased only slightly during the sprouting process. Total dry matter was reduced only 5% during the seven day sprouting period.

Table 3. Effects of sprouting on carotene content of five classes of wheat in vitamin A equivalents/lb.

	Days of Sprouting		
Class	0	3	7
Hard Red Spring	300	300	400
Soft White	300	500	500
Soft Red Winter	400	400	400
Durum	300	400	400
Hard Red Winter	100	400	600

The marketing study has shown there to be a limited demand for wheat sprouts, especially among the younger consumers. Holidays and university vacations reduced this demand. Advertising and recipes would have been a big help in the promotion of wheat sprouts. Shelf life was found to be about seven days under refrigeration. At room temperature, the sprouts turned brown in a day or two. In most situations the sprouts were delivered to the store on Friday afternoon and they were all sold by Saturday night. One pound of wheat produced almost two pounds of sprouts (seven or eight 4-oz packages). The 4 oz packages were sold wholesale for 19¢ each and retailed at 25-38¢ each. Retail price did not seem to affect the demand. The labels were a disaster because they were easily torn off or crumpled, making for an unattractive display.

Wheat sprouts have a bland, slightly sweet taste, making them useful in many dishes. In some dishes they have a mild nut-like flavor. Wheat sprouts were found to fit well into salads, casseroles, oriental dishes and some soups and sandwiches. They work well in chili without the problem associated with beans. They should be chopped or ground to be used in meat loaf or as a meat extender. Wheat sprouts may be used as an alternative for rice. In cookies and candies, they tend to dry out and become hard as a rock. Wheat sprouts have a mild malt flavor if dried and ground into a flour.

The egg rolls containing wheat sprouts were well received by most consumers (Figure 2). Out of 150 respondents, two individuals ranked the egg rolls as very poor. In contrast, 65 ranked the taste as excellent. The average response ranked the egg rolls in the good-excellent range for taste and aroma, with the inside appearance as good (Table 4).

Figure 2. Consumer acceptance of wheat sprout egg rolls by taste, aroma and inside appearance.

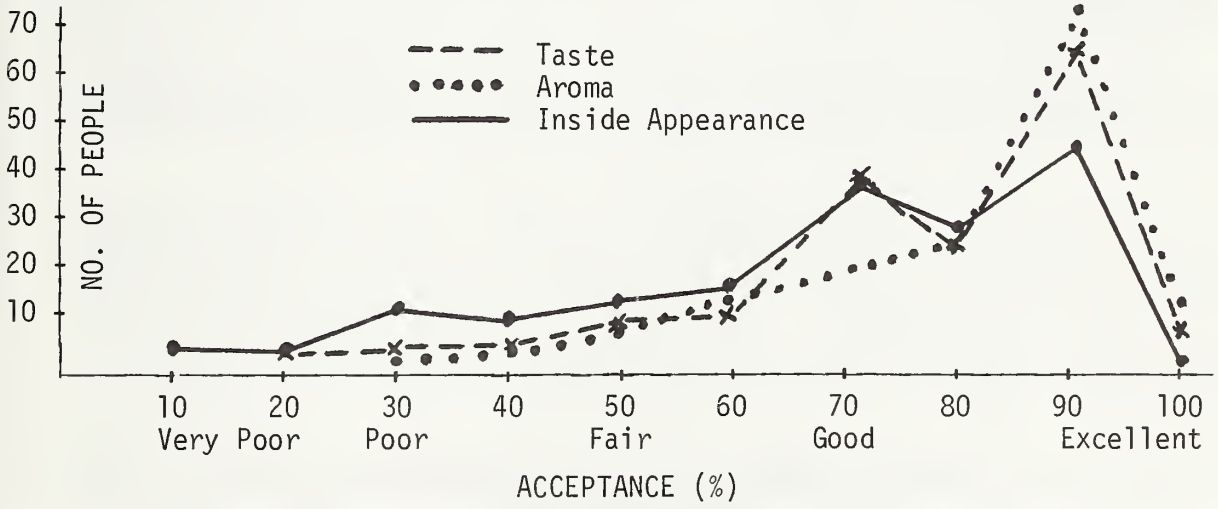


Table 4. Average ranking and standard deviation of consumer response to egg rolls containing wheat sprouts.

	%	Std. Dev.	Std. Error	Appearance
Taste	83.45	15.25	1.24	Good
Aroma	80.96	15.63	1.28	Good
Inside Appearance	72.81	21.11	1.72	Good

HIGH FIBER BREAD CONTAINING BREWERS' SPENT GRAIN

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During the mashing stage of brewing the major constituents of malt and a carbohydrate adjunct, such as corn grits or rice, are subjected to enzyme-catalyzed hydrolysis. The solid residue (spent grain) after filtering the mash, is mainly husk, bran, and embryo residues of the malt kernel, and corn bran if corn grits were used as adjunct.

The wort is hopped and then boiled to extract flavoring constituents from the hops and to increase the specific gravity of the wort. During this boiling a complex precipitate called "trub" forms which consists mainly of proteins, phenolic compounds, and lipids. This trub and the solid hop residue are removed and is usually added to the spent grain for disposal; the cooled, clear wort is inoculated with yeast for the fermentation process.

The spent grain-hop-trub mixture, currently about 700,000 tons dry weight annually in the United States, is one of the main by-products of brewing, and has traditionally presented a disposal problem (1).

Recently interest has developed in dietary fibrous materials which may play a role in the prevention of certain noninfectious diseases such as diverticulosis, colon cancer, hemorrhoids, arteriosclerosis, varicose veins, and appendicitis (2). Since spent grain is high in fiber as well as protein, it may have useful application in human nutrition. This report describes the functionality and consumer acceptance of brewers' spent grain in bread.

Chemical and Physical Analyses of Flours and Brewers Spent Grains (BSG)

The composition of the various flours and flour constituents are given in Table I. The protein of flours containing 5, 10, and 15% BSG is increased by 4.3, 9.8 and 15.2% respectively compared with the 30% whole wheat control.

For advantage to be taken of increases in crude fiber or acid detergent fiber relative to the 30% whole wheat flour control, a minimum of 10% replacement of white flour by BSG would have to be used.

Table 1. Percent Composition of BSG and Flours[1/]

	Protein[2/]	Ash	Crude Fiber	Crude Fat	Acid Detergent Fiber	Cellulose	Lignin
BSG	34.4	3.6	14	8.3	36	10	17
White Flour	16.5	0.41	0.05	1.3	--	--	--
Whole Wheat Flour	16.8	2.1	2.0	2.4	7	--	--
95% White, 5% BSG	17.1	0.56	0.7	1.7	2	0.5	0.9
90% White, 10% BSG	18.0	0.73	1.4	2.0	4	1.0	1.7
85% White, 15% BSG	18.9	0.88	2.1	2.3	6	1.5	2.6
70% White, 30% Whole Wheat	16.4	0.92	1.0	1.6	2	--	--

1/ Values are on a dry basis
2/ Calculated on the basis of wheat protein = Nx5.7 and BSG protein = Nx6.25

The particle size-nitrogen distribution of the milled BSG is shown in Table II. The largest portion of the material was retained on the 65 mesh sieve, and, as with that retained on the 32 and 80 mesh sieves, contained a lower concentration of nitrogen than the parent material; these fractions appeared to be mainly fibrous husk tissue. The finer fractions (150, 200, and thru 200 mesh) were higher in nitrogen by 1.25 to 1.5-fold than the parent and likely contain a high proportion of aleurone and embryo tissue. For example, the protein level (Nx6.25) of the fraction retained on the 200 mesh sieve is 46%; this fraction would increase crumb protein significantly if 5 to 10% of the white flour were substituted with it.

Table II. Yield and Nitrogen Levels of BSG Fractions

Sieve Mesh/in	32	65	80	100	150	200	Thru 200	Parent
Fraction Yield, %	1.4	40.5	8.0	13.8	13.7	14.7	7.9	100
N, % Dry Basis	5.1	4.8	5.1	5.8	6.8	7.4	8.0	5.5

Table III shows the amino acid composition of the parent BSG in comparison with the amino acid levels of white flour and whole wheat flour. The protein level of the BSG was about twice that of the wheat flours and this relationship existed for most of the constituent amino acids, except for glutamic acid, methionine, glycine, arginine and cystine.

Table III. Amino Acid Composition

	g Amino Acid/100 g Sample (Dry Basis)		
	White Flour	BSG	Whole Wheat Flour
Lysine*	0.41	1.00	0.58
Histidine*	0.43	0.98	0.50
Ammonia	0.78	1.14	0.78
Arginine*	0.72	1.59	1.01
Aspartic Acid	0.85	2.04	1.14
Threonine*	0.47	1.20	0.55
Serine	0.75	1.54	0.80
Glutamic Acid	6.03	8.36	5.88
Proline	2.36	4.22	2.18
Half Cystine	0.24	0.35	0.24
Glycine	0.64	1.18	0.79
Alanine	0.54	2.17	0.69
Valine*	0.88	2.03	0.96
Methionine*	0.49	0.75	0.45
Isoleucine*	0.68	1.45	0.70
Leucine*	1.25	3.93	1.31
Tyrosine	0.49	1.35	0.55
Phenylalanine*	0.91	2.04	0.93
N Recovery %	100	91.6	105

*Essential Amino Acids

Physical Dough Properties

Table IV shows farinograph data for the hard red spring wheat flour, the 30% whole wheat flour and the flours containing 5 and 10% BSG. The absorption of the flour blends containing the BSG increased as the level of BSG increased. The dough developing time was similar in all cases, whereas with the higher level of BSG the stability of the dough increased. The flour blend containing the 30% whole wheat flour showed a reduction in dough developing time and stability.

Bread Baking

Based on preliminary studies with "pup" loaves we decided to produce for the preliminary taste panel one-lb loaves of bread made with flours containing 5 and 10% unheated BSG and BSG dried at 45°, 100° and 150° C. The 30% whole wheat bread had color and other physical characteristics similar to the bread containing the BSG. Table V shows typical effects of BSG on specific loaf volume.

Table IV. Farinograph Data for Flour and Flour Blends

Flour	Absorption[1]	Dough Developing Time min.	Stability min.
White Flour	62.6	6.5	12.5
30% Whole Wheat	65.4	5.0	9.0
5% BSG[2]	65.0	6.5	12.5
10% BSG[2]	65.6	6.5	15.0

1/ 14% moisture basis
2/ No additional heat treatment

Table V. Loaf Volume Decrease[1]

BSG Drying	BSG %	Specific Loaf Volume Decrease %
None	5	0
	10	11.4
	15	17.0
100° C	5	5.7
	10	11.6

1/ Average of 20 loaves (1 lb) relative to 30% whole wheat control.

Organoleptic Evaluation

Preliminary Panels

These small panels provided an estimation of the levels of flour replacment by BSG and the drying treatment of the BSG that would be chosen for breads to be supplied for the larger panel (consumer panel).

Bread was evaluated on a scale of 1 to 7 for: crumb texture, mouthfeel, flavor intensity, off-flavor, and overall preference (3). Values of 1 to 7, used for variance analysis, corresponded to increasing intensity or desirability as follows:

Crumb texture; 1 = smooth and moist, 7 = coarse and dry
Mouthfeel; 1 = nonabrasive, 7 = abrasive
Flavor intensity; 1 = mild, 7 = pronounced
Off-flavor; 1 = imperceptible, 7 = pronounced
Overall preference; 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = like slightly, 5 = like moderately, 6 = like very much, 7 = like extremely

A numerical value of 3.5 was taken as the lowest level of acceptability for overall preference.

Consumer panels

Panel members received a quarter-slice of the whole wheat control and a quarter-slice of BSG bread. One-half ounce portions of sweet cream, salted (2%) butter were served to the panelists as a free-choice condiment for the bread samples. Overall preference was determined as for the preliminary panel.

Evaluation by the Preliminary Panel

Table VI shows the evaluation of breads made from BSG with various drying treatments at the 5% level of incorporation in flour. Crumb texture was less coarse than that of the 30% whole wheat bread control, and in some cases there was less abrasive mouthfeel. Bread containing BSG treated at 150° C appeared to be somewhat less flavorful than the whole wheat control bread. Off-flavor was not a problem. The breads containing 5% BSG replacement of flour had the same overall preference as the control regardless of heat treatment.

At the 10% level of substitution, however, (Table VII) the BSG showed deleterious effects relative to the 30% whole wheat control, particularly the BSG which had been dried at 150° C. In this case crumb texture was more abrasive. Flavor intensity was increased, and this was judged to be an off-flavor. The 150° C-treated material reduced overall acceptability. It is evident that there was no advantage in treating BSG at high temperatures. Similarly, substitution at the 10% level by BSG treated at 100° C seemed undesirable. While this did not change crumb texture or mouthfeel, flavor intensity was increased and this was judged to be an off-flavor which resulted in significantly decreased overall preference. BSG with no additional heat treatment and BSG treated at 45° C at the 10% level showed no significant differances in any category, except for an inexplicably lowered overall preference for the BSG with no additional heat treatment.

Evaluation by Consumer Panels

The results from the preliminary panels indicate that additional heat treatment of commercially dried BSG is of doubtful value and that 10% substitution of flour with BSG is probably the upper limit of

Table VI. Panel (N=29) Evaluation of 30% Whole Wheat Bread and BSG Bread at 5% Substitution Level

Flour Type and BSG Drying Treatment	Sample Attribute and Mean Scores				
	Crumb Texture	Mouthfeel	Flavor Intensity	Off-Flavor	Overall Preference
30% Whole Wheat	4.50[a/]	4.65[a/]	4.26[a/]	3.02[a/]	3.91[a/]
BSG 45° C	3.49[b/]	3.20[b/]	3.05[b/]	2.36[b/]	4.20[a/]
30% Whole Wheat	3.92[a/]	3.61[a/]	3.26[a/]	2.28[a/]	4.57[a/]
BSG None	3.37[b/]	3.35[a/]	3.27[a/]	2.63[a/]	4.25[a/]
BSG 100° C	3.52[b/]	3.43[a/]	3.41[a/]	2.61[a/]	4.44[a/]
30% Whole Wheat	4.51[a/]	4.30[a/]	4.04[a/]	2.85[a/]	4.01[a/]
BSG 150° C	3.83[b/]	3.42[b/]	2.89[b/]	2.44[a/]	4.11[a/]

a,b/ Mean scores within an experiment in the same column with the same superscript are not significantly different at the 5% level.

Table VII. Panel (N=26-29) Evaluation of 30% Whole Wheat Bread and BSG Bread at 10% Substitution Level

Flour Type and BSG Drying Treatment	Sample Attribute and Mean Scores				
	Crumb Texture	Mouthfeel	Flavor Intensity	Off-Flavor	Overall Preference
30% Whole Wheat	4.01[a/]	4.12[a/]	3.22[a/]	2.88[a/]	3.82[a/]
BSG 45° C	3.85[a/]	3.63[a/]	3.47[a/]	2.76[a/]	3.87[a/]
30% Whole Wheat	3.76[a/]	3.55[a/]	3.52[a/]	2.36[a/]	4.40[a/]
BSG 100° C	3.90[a/]	3.85[a/]	4.39[b/]	3.13[b/]	3.67[b/]
30% Whole Wheat	4.00[a/]	3.86[a/]	3.32[a/]	2.48[a/]	4.15[a/]
BSG None	4.16[a/]	3.95[a/]	3.59[a/]	2.95[a/]	3.44[b/]
BSG 150° C	4.74[b/]	4.51[b/]	4.04[b/]	3.94[b/]	2.86[c/]

a,b,c/ Mean Scores within an experiment in the same column with the same superscript are not significantly different at the 5% level.

substitution. Accordingly, the consumer panels were presented only bread containing BSG without additional drying and with BSG treated at 100° C, both at 5 and 10% levels of flour substitution. In addition, unheated BSG was evaluated at the 15% level of flour substitution.

From the consumer panels (Table VIII) it is apparent that the bread with the BSG at the 5 and 10% levels had the same acceptability as the whole wheat control. Furthermore, there was no advantage in heating the commercially dried BSG. At the 15% level of BSG incorporation there was a significant decrease in consumer acceptability. The use of BSG in bread at the level of 5 and 10% flour replacement may be practicable, particularly if consumers are aware of the possible benefits of the increased fiber and protein levels. Improvement in the performance of BSG may be possible by proper selection of particle size.

Table VIII. Panel (N=194-210) Evaluation of 30% Whole Wheat Bread and BSG Breads at 5, 10 and 15% Levels of BSG

	BSG Heat Treatment	Substitution Level	Mean Score Overall Preference
30% Whole Wheat	-	-	5.25[a/]
BSG	None	5%	5.14[a/]
30% Whole Wheat	-	-	5.05[a/]
BSG	100C	5%	5.03[a/]
30% Whole Wheat	-	-	5.12[a/]
BSG	None	10%	5.20[a/]
30% Whole Wheat	-	-	5.19[a/]
BSG	100C	10%	5.18[a/]
30% Whole Wheat	-	-	5.24[a/]
BSG	None	15%	4.94[b/]

a,b/ Mean scores within an experiment in the same column with the same superscript are not significantly different at the 5% level

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NBS STANDARD REFERENCE MATERIALS 1567, WHEAT FLOUR, AND 1568, RICE FLOUR, CERTIFIED FOR CONCENTRATIONS OF SELECTED TRACE ELEMENT NUTRIENTS AND ENVIRONMENTALLY IMPORTANT CONSTITUENTS

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The National Bureau of Standards has issued two unfortified flour Standard Reference Materials--a bleached wheat flour and a rice flour. The Certificates of Analysis for SRM 1567, Wheat Flour, and SRM 1568, Rice Flour list concentration values for selected trace element nutrients, environmentally important elements, and other trace elements of undefined function. These SRM's were developed because of the importance of trace elements, which are present in foods at or below the parts-per-million level and because of the difficulty of determining them reliably. The certified reference materials are intended primarily for evaluating the accuracy of these elemental determinations in flours and other cereal foods. They will be useful for developing reliable trace element methods, for calibrating the instrumentation used in these analyses, and for providing certified values to which experimental data acquired at different times by the same or different laboratories can be compared.

Introduction

The chemical composition of cereal foods is of interest to environmentalists, medical researchers, nutritionists and other life scientists because of the large consumption of such foods throughout the world. For example, the U. S. population obtains approximately one-fourth of its total caloric content from cereal foods, much of which is consumed as refined flour in bread or other bakery products.

Although present in foods at concentration levels of parts-per-million and below, a number of trace elements are important as essential nutrients, others as undesirable contaminants, while still others have as yet undefined roles. The Food and Nutrition Board of the National Academy of Sciences has established Recommended Dietary Allowances for three trace element nutrients: iron, zinc, and iodine (1). It is expected that in 1978 the Board will announce RDAs, as provisional ranges, for six more: selenium, copper, manganese, chromium, molybdenum, and fluorine.

The functions, metabolism, and analytical determinations of trace elements have been described (2, 3, 4). In their papers, both W. Mertz and W. Wolf recommend the use of more than one method and comparison of results against certified reference materials to minimize the possibility of systematic errors in the results. However, the use of more than one method may not be feasible for the life scientist who has neither the independent analytical instrumentation at his disposal nor the time to conduct these independent evaluations.

Moreover, the selection of an analytical method of known reliability may not be possible. As R. H. Matthews (5) observed, "The need for reliable standardized methods of analysis is acute for many foods and nutrients." She cites discrepant data for magnesium as evidence that methodology is of concern in mineral element determinations.

In most analytical procedures for the determination of trace elements, the initial step is usually dry or wet ashing the material. Each technique has certain disadvantages. Dry ashing results in losses of volatile elements, such as selenium, while wet ashing can yield high results because of impurities in the acids. However, wet ashing with high-purity acids, such as those purified by sub-boiling distillation can limit the amount of these impurities to acceptable levels (6). After ashing the sample, the next step is the preparation of synthesized standards which simulate the matrix of the ashed sample in solution. These standards have graded concentration levels of the trace elements being determined.

The trace elements in the sample solution are determined instrumentally in a comparative mode, that is by comparing the signal produced by the trace element of interest in the sample to the signals produced by the known amounts of the same element in the synthesized standards. It is obvious that if the standards are in error, the results for the sample will be inaccurate. The use of chemical enrichment procedures for improved sensitivity also increases the possibility of contamination.

The use of reference materials with certified concentrations of trace elements as one of the samples being analyzed serves to validate the methodology and the techniques.

The purpose of this paper is to describe the development and certification of two flour Standard Reference Materials--SRM 1567, Wheat Flour, and SRM 1568, Rice Flour.

Sources of Flour Materials

The wheat flour was described by the supplier as milled from a blend of Hard Red Spring and Hard Red Winter Wheat grown primarily in South Dakota. It was bleached and brominated in accordance with standard treatments for commercial bakery use.

The rice flour was described as 100% long grain from Arkansas.

Processing of Materials

Each flour material was processed in the following manner. Approximately 225 kg of the flour was passed through a sieve with openings of 425 μm (No. 40) for removal of any possible foreign matter. After sieving, the flour was blended in a stainless steel V-blender for one hour. The blended flour was dispensed

into bottles which were numbered sequentially and sealed. The bottled samples were the irradiated with 2.5 megarads of Co-60 radiation for microbiological control.

Homogeneity Testing

A preliminary evaluation of elemental homogeneity was made by instrumental neutron activation. Samples weighing 150 to 300 mg, in duplicate, were taken from sixteen bottles selected at random from the series of sequentially numbered bottles. The activities, corresponding to the radionuclides of Mn, K, Zn, Na, and Br, were counted. The homogeneity of other certified elements was evaluated using samples of 400 mg or less with the exception of mercury and calcium for which 500 mg and 1 g, respectively, were used.

Methodology and Results

The following analytical methods were used at NBS to characterize the materials:

1. Atomic absorption spectrometry
2. Flame emission spectrometry
3. Isotope dilution, spark source mass spectrometry
4. Neutron activation, instrumental
5. Neutron activation, radiochemical
6. Polarography

Cooperative analyses on these candidate SRM's for copper and zinc were performed at the Nutrition Institute, Nutrient Composition Laboratory, Beltsville, Md. 20705.

Table 1 lists the certified concentrations of elements determined in SRM 1567, Wheat Flour. They are based on the agreement of results by at least two independent analytical methods and are reported on a "dry-weight" basis. At least 400 mg of the SRM should be used by an analyst to obtain concentration values within the uncertainty limits of the certified concentrations

Except for selenium and mercury, elements may be determined on samples of this SRM that have been vacuum-dried at approximately 25 ¼C for 24 hours at a pressure not greater than 70 Pa (0.5 mm Hg) with a cold trap at a temperature of about 30 °C or below. Selenium and mercury should be determined on undried samples; other elements may be so determined. However, because the Certificate values are reported on a 'dry-weight' basis, the elemental concentrations should be adjusted for the moisture content of the samples. The moisture content, which was approximately 9% when bottled, should be determined by either the vacuum-drying procedure previously described or drying the sample in air in an oven at 85 °C for 24 hours. Both of these procedures yielded the same loss in weight. Samples for analysis should not be oven-dried because elements may be lost by volatilization.

The estimated uncertainty of the concentration value is based on judgment and represents an evaluation of the combined effect of method imprecision, possible systematic errors among methods, and material variability for samples 400 mg or more. No attempt was made to derive exact statistical measures of impression because several methods were involved in the determination of the constituents.

The elemental concentrations in SRM 1567, which are listed in Table 2, are not certified but are included for information only. They were not certified because they were not based on the agreement of results by two or more independent analytical methods. These values are also based on the "dry-weight" of material.

Similarly for Rice Flour, SRM 1568, Tables 3 and 4 list the certified and uncertified concentrations, respectively, of the elements. These values are also based on a minimum sample size of 400 mg and are reported on a "dry-weight" basis.

A comparison of the elemental concentrations present in the two flour SRM's show significant differences for such elements as arsenic, selenium, zinc, iron, manganese, and molybdenum. These differences should make it possible for analysts to validate their analytical curves of instrumental response versus concentration at two different concentrations.

Discussion

The U. S. Food and Drug Administration requested and partially supported the development of these SRM's. They were developed primarily as trace element nutrients and contaminants.

Further characterization of these SRM's will continue. As more data is accumulated at NBS and at cooperating laboratories, the Certificates of Analysis will be revised to reflect this additional information.

Other SRM's of interest to nutritionists, and life scientists are available from NBS (7). Examples of these are Spinach and Brewers Yeast. We welcome your suggestions for additional SRM's.

Table 1. Certified Values of Elements in Wheat Flour, SRM 1567

Minor Constituents

Element	Content Wt. Percent
Potassium	0.136 ± 0.004
Calcium	0.019 ± 0.001

Trace Constituents

Element	Content µg/g	Element	Content µg/g
Iron	18.3 ± 1.0	Copper	2.0 ± 0.3
Zinc	10.6 ± 1.0	Selenium	1.1 ± 0.2
Manganese	8.5 ± 0.5	Cadmium	0.032 ± 0.007
Sodium	8.0 ± 1.5	Mercury	0.001 ± 0.0008

Table 2. Non-certified Values for Elements in Wheat Flour, SRM 1567

Element	Content µg/g	Element	Content µg/g
Bromine	(9)	Nickel	(0.18)
Rubidium	(1)	Arsenic	(0.006)
Molybdenum	(0.4)	Tellurium	(≤0.002)

Table 3. Certified Values of Elements in Rice Flour, SRM 1568

Minor Constituents

Element	Content Wt. Percent
Potassium	0.112 ± 0.002
Calcium	0.014 ± 0.002

Trace Constituents

Element	Content µg/g	Element	Content µg/g
Manganese	20.1 ± 0.4	Arsenic	0.41 ± 0.05
Zinc	19.4 ± 1.0	Selenium	0.4 ± 0.1
Iron	8.7 ± 0.6	Cadmium	0.029± 0.004
Sodium	6.0 ± 1.5	Cobalt	0.02 ± 0.01
Copper	2.2 ± 0.3	Mercury	0.0060±0.0007

Table 4. Non-certified Values for Elements in Rice Flour, SRM 1568

Element	Content µg/g	Element	Content µg/g
Rubidium	(7)	Nickel	(0.16)
Molybdenum	(1.6)	Tellurium	(≤0.002)
Bromine	(1)		

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PREPARATION OF SOY BREAD UTILIZING LOW PROTEIN WHEAT FLOUR

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The protein fortification of wheat flour breads with soy flour is becoming increasingly important in the areas of the world which lack sufficient animal protein resources. The use of soy flour in the white pan bread formulation tends to reduce the bread-making potential of the wheat flour. Consequently, technological measures or/and use of dough strengtheners are necessary to produce acceptable breads. Published studies evaluated types,levels, granulations, and forms of soy flours, the types and levels of surfactants and oxidants, and many of the production variables. None of them considered the use of flour that is quantitatively low in protein, although this type of flour is frequently encountered in various parts of the world.

The objective of this investigation was to evaluate the bread-making performance of a wheat flour defined as low protein or less than 11.0% protein (N x 5.7, 14% m.b.). Conditions specifically designed to optimize the production of soy bread were developed to produce doughs of acceptable mechanical properties and breads of high-quality characteristics.

Methods and Materials

Experimental approach. The tests were conducted at experimental and production levels. The first stage was used to identify the important technological factors in soy bread production, and the second one to verify the small-scale observations in a full-scale operation.

Bread formula and procedure. Table I details the basic formula which was selected at the beginning of the study and was altered as required by the test variables. The main operational conditions are also indicated in this Table. Note that the method used was a sponge and dough procedure and that the soy flour was added as an ingredient, at a rate of 12% on the flour basis.

Variables investigated. Six conditions given below (Table II) were studied at an experimental level, and conditions (2), (3), (5), and (6) also on a commercial scale. The variable (7) was evaluated in the full-scale operation only.

Flour. The flour used was derived from a single hard red winter wheat blend and was commercially milled at different days. The protein content ranged from 9.8% to 10.7%, averaging 10.32% (N x 5.7; 14% m.b.); its ash values were 0.55-0.45% (14% m.b.).

Table I

SOY BREAD FORMULA

	Ingredient	%
Sponge:	Flour	50
	Yeast	3
	M.y.f.	0.25
	H_2O	60
Dough:	Flour	50
	Salt	2
	Sugar	6
	Shortening	3
	Soy	12
	H_2O	Variable

Procedure:

1. Sponge fermentation time - 3 hours at 80°F.
2. Dough: temperature 80°F, floor time - 25 minutes, intermediate proof - 11 minutes. Proofed to template (about 70 minutes) at 100°F. and 80-86% r.h.
3. Baking: 22 minutes at 425°F.

Table II
Variables tested:

(1) sponge/dough ratio

(2) point and form of soy flour addition

(3) mixing

(4) fermentation -proof-floor times

(5) oxidants, types and levels

(6) surfactants

(7) amylases

Oxidants. Potassium bromate, ascorbic acid (AA), and ADA were added singly and also in combinations.

Surfactants. Sodium-stearoyl-2-lactylate (SSL), ethoxylated mono- and diglycerides, mono- and diglycerides, succinylated monoglycerides, diacetyltartaric acid ester, Super Do (blend of succinylated monoglycerides and distilled monoglycerides), and Tanden 8 [blend of mono-acid diglycerides with polysorbate 60 (40%)] were tested on experimental scale, and SSL, Super Do, and Tanden 8 in commercial-size experiments.

Soy flour. A defatted soy flour (Nutrisoy, product of ADM), containing 52.4% protein (N x 5.7, 5.0% m.b.) was used.

Results and Discussion

Experimental baking tests:

One thousand grams of flour per batch were used in this experimental series. The mixing step was carried out in a Hobart-120 mixer, equipped with a McDuffy bowl and fork.

Effect of sponge/dough ration and of manner of soy addition. The ratios of 50/50, 60/40, 70/30, and 100 were tested. The points and forms of the soy flour addition were investigated for each sponge/dough ratio. The soy flour was either incorporated early by blending it into the flour along with other dry ingredients, or added in hydrated form, or blended in dry late (after the wheat flour dough had been developed). Of these conditions best results were obtained with a 50/50 sponge/dough ratio and the late soy flour addition.

Oxidants. Potassium bromate was superior to AA and ADA when the oxidants were used singly. However, combinations performed better than the individual oxidatns. The best combination for the flour used was a blend of potassium bromate and AA, in proportions of 15 ppm ($KBrO_3$) and 250 ppm AA. Specific volume of bread increased from 4.40 (no oxidant) to 5.24 ml/g (proper oxidation).

Surfactants. The use of different agents at varied levels resulted in an additional improvement. The spec. volume of bread loaves increased from 5.24 to a range of 5.43-5.5 ml/g. In general this system required a low level of emulsifier--about 0.25% (flour basis). Most of the types tested performed equally well in this system. The SMG and polyoxy-ethylene sorbitan monostearate were perhaps slightly superior to others.

Production tests:

It is well known that the procedures successful at a small scale are not necessarily transferable to a full-scale commercial operation without some modifications. The production variables may be more or less critical and the dough characteristics may respond differently to the large-scale handling equipment than to small-scale handling. Consequently, our experimental data needed to be confirmed under production conditions.

Production equipment. The tests were carried out in the commercial-size bakery of the AIB. The mixing was conducted in a vertical mixer of 160 quart capacity using 30 lbs. flour per batch or in a horizontal Readko mixer of 300 lbs. total capacity equipped with low (49 rpm) and high (98 rpm) speeds. The amount of flour per batch used in the horizontal mixer was 100 lbs. The doughs were divided in a two-pocket variable speed divider,rounded in a conical rounder, given an 11 minute intermediate proof in an overhead proofer, passed through a cross-grain sheeter-molder, and baked in an indirect gas-fired single lap over.

Type of mixer. Difficulties were encountered with a vertical mixer. They were due to a poor incorporation of the soy flour into the wheat flour-soy system.

The doughs were not homogenous and smooth as desired, and lumping of a varied degree became a problem in most cases. To remedy or at least minimize this deficiency, the soy flour was incorporated according to various schedules, and also the mixing speeds were varied. Dusting presented a serious difficulty with the vertical mixer when the soy flour was added in a dry form. Thus, the only possibility of using this type of mixer is to add the soy flour early along with other dry ingredients. This method, however, produced breads of inferior quality than when soy flour was added at a later stage.

Soy flour addition and horizontal mixing. Since we found the vertical mixer to be unsuited, further tests were conducted with a horizontal mixer. Various procedures of soy addition were tested, as eviden from Table III. On the basis of these data the dry soy late addition was selected as optimal for further work. These results are in a general agreement with the small-scale observations.

Table III

Soy Addition

	Bread Characteristics[a]				
	External		Internal		Total
	Sp. Vol.	Break & Shred	Grain	Texture	
Dry Soy - early addition	4.97	P	G-	G	32
Dry Soy - late addition	5.08	P	VG-	G	37
Hydrated Soy - late addition	5.09	P	G	G	35
Dry Soy with Water - late addition	5.25	P	G	G+	36

(a) quality rating scale: excellent, very good, good, fair, poor.

Effects of oxidation. Table IV illustrates the importance of oxidants. It is obvious that oxidation per se is a necessary factor in preparation of soy bread. Where oxidants were used singly, potassium bromate performed better than the other two (AA, ADA). All individually used oxidants were inferior to the combination of ascorbic acid and potassium bromate. The optimal proportion of these two oxidants for the flour used was: 250 ppm of AA and 60 ppm potassium bromate. The values in parentheses in this table are loaf volumes obtained in experimental tests. It can be readily observed that the values of the commercial run paralleled those of the experimental baking.

Table IV

Effect of Oxidation

Treatment	Bread Characteristics[a]				
	External		Internal		Total
	Sp. Vol.[b]	Break & Shred	Grain	Texture	
Control	4.90 (4.42)	P	VG-	G	37
AA-75ppm	4.81 (4.80)	P	G-	G-	33
$KBrO_3$-60ppm	5.16 (4.81)	P	G-	G	34
ADA-60ppm	4.89 (4.86)	P	F	G	29
AA-250ppm $KBrO_3$-60ppm	5.61 (5.09)	F	G	G	37
AA-250ppm	5.51 (5.16)	P	F+	G	31

(a) quality rating scale: excellent, very good, good, fair, poor.

(b) values in parentheses indicate loaf volume in experiment bakery.

Effects of surfactants (Table V). The flours in this series received a proper degree of oxidation (250 ppm AA-60 ppm bromate). The use of surfactants produced additional improving effects. The best use-level of the surfactant appeared to be 0.5% (flour basis), at which there was little difference between SSL and Super Do while Tandem 8 produce slightly larger loaves of higher quality.

Table V

Effect of Surfactants

Treatment	Bread Characteristics[a]				
	External		Internal		Total
	Sp. Vol.	Break & Shred	Grain	Texture	
Control	5.01	P	G	G	35
SSL, 0.25%	5.36	F-	G	G+	37
0.50%	5.41	F-	G	G+	37
Super Do, 0.25%	5.06	F-	G-	G	35
0.50%	5.33	F-	G-	G	37
Tandem 8, 0.50%	5.56	G-	G	G+	40
0.50%	5.57	G-	G-	G+	39

(a) quality rating scale: excellent, very good, good, fair, poor.

Effect of amylases. The flours used up to this point were intentionally unmalted because we thought that the enzymatic activity of malt may tend to reduce the strength of the already weak flour. However, the data shown in Table VI are contrary to this expectation. Both cereal amylases (malt) and the fungal preparation (Do-Tone) tested, produced improvements when added at proper levels. The combined effects of SSL and malt or fungal amylases were more prominent than the effect of the enzymes only. The fungal preparation in combination with the surfactant was somewhat less beneficial than the combination of malt-SSL.

Table VI

Effect of Amylases

Variable	Bread characteristics[a]				
	External		Internal		Total
	Sp. Vol.	Break & Shred	Grain	Texture	
Unmalted Flour[b]	5.38	F	G	G	37
Unmalted Flour & Malt (50g/cwt)	5.77	G	G	G+	42
Unmalted Flour & Malt (250g/cwt)	5.76	F	G	G	38
Unmalted Flour & Fugal Amylase (1.25g/cwt)	5.79	G	G	G+	43
Unmalted Flour & Fugal Amylase (6.25g/cwt)	5.42	F	G-	G	36
Unmalted Flour +0.25% SSL	5.69	G	G+	G+	42
Unmalted Flour & Malt (50g/cwt) & 0.25% SSL	6.09	G	G+	G-	40
Unmalted Flour & Fugal (1.25/cwt) & 0.25% SSL	5.88	G	G+	G+	40

(a) quality rating scale: excellent, very good, good, fair, poor.

(b) flours contained optimal levels of oxidants (a.a & $KBrO_3$).

In the summary table (Table VII) the results with soy bread are presented in terms of white pan breads. The flour used in these experiments produced loaves of white pan bread with a spec. volume of 6.22 ml/g when our test formula using optimum malting, oxidation, and a sponge/dough ratio of 70/30 was employed. This

indicates the maximum bread-making potential of the flour. When this white pan bread formula was used for soy bread preparation without any adjustments, except absorption, a 15% reduction in volume was observed. The following modifications caused improvements: (a) change of the sponge/dough ratio to 50/50 and selection of proper oxidants (13% drop vs. white bread), (b) further, addition of malt (6% drop vs. white bread), and (c) addition of SSL or other surface active agent (2% drop vs. white bread.

Table VII

SUMMARY

Variable	Spec. Vol.	S.D.	% of Control
WHITE PAN BREAD: sponge/dough (70/30) optimum oxidation (15ppm $KBrO_3$) optimum malt (50g/cwt)	6.22	0.098	100
SOY BREAD: sponge/dough (70/30) 15ppm $KBrO_3$ 50g/cwt malt	5.29	0.081	85.0
sponge/dough (50/50) no oxidants, no malt	5.08	0.079	81.7
sponge/dough (50/50) AA-250ppm, $KBrO_3$-60ppm	5.40	0.56	86.8
sponge/dough (50/50) AA-250ppm, $KBrO_3$-60ppm 50g/cwt malt	5.82	0.052	93.6
sponge/dough (50/50) AA-250ppm, $KBrO_3$-60ppm 0.25% SSL 50g/cwt malt	6.09	0.152	97.9

To conclude: The presented results show that the steps necessary to optimize the soy bread production by a sponge and dough process are: (a) late addition of soy flour, (b) use of a proper type, level, and combination of oxidants, (c) adjustment of the sponge/dough ratio to 50/50, (d) normal malting with malt or fungal amylases, and (e) use of a selected surfactant. Under these conditions, a loaf volume comparable to that of white bread from the wheat flour used can be expected.

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PEA: A HIGHLY FUNCTIONAL FORTIFIER IN WHEAT FLOUR BLENDS

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Introduction

As world population and monetary inflation increases, food deficiency becomes more acute in terms of quantity and quality. The problem of malnourishment is often a shortage of protein in human foods. Efforts have been made to improve wheat, the major food cereal for protein and nutritional quality, through breeding and fortification of wheat flour with high-protein, high-lysine material to make a more nutritious low-cost food.

The main limitation of wheat protein is its inadequate balance of essential amino acids; lysine, methionine, threonine and isoleucine. Researchers, for several years, have placed emphasis on the development of high-protein type bread products. The use of legume flours (particularly soy) has been extensively studied as a fortifier to improve the nutritional value of bread (Bean, Fellers, Tsen, and others) (1-7). Soy flour is an effective additive, not only because of its high protein content, but also because of its higher lysine content to complement wheat flour.

The use of other legume flours to improve the nutritive value of bread has recently received considerable interest. To review a bit some of the more recent work - the production of high-protein bread from wheat-faba bean composite flours was studied by McConnel et al. (8). They found that addition of faba bean to hard red spring wheat flour at the rate of 10, 20, 30, and 40% resulted in a progressive decrease in loaf volume and a deterioration in crumb grain even with addition of a dough conditioner such as sodium stearoyl-2-lactylate (SSL). However, these difficulties could be overcome and protein levels markedly increased by using faba bean concentrate prepared by air classification in place of faba bean flour. D'Appolonia (9) studied baking potential of several legume flours (faba beans, pinto beans, navy beans, mung beans, and lentils). He too found a loss in breadmaking characteristics as the level of legume flour was increased, but could be made to work with the addition of SSL, higher oxidation, and less fermentation time at the lower levels of 5 and 10%. Thompson found breads with mung bean protein isolate at the 10% level were acceptable.

Similarly, Khan et al. (10) found a peanut protein concentrate (PPC) at 10% level acceptable in baking and superior in flavor and color to a full fat soy flour.

The use of raw and cooked flour from yellow peas (Pisum sativum), an important legume grown largely in the Pacific Northwest area of the U.S. and

Canada has been investigated only to a limited extent as a supplement in bread baking. Dried peas have been widely used as a source of protein in low cost diets principally as gruels or soups. They are also valuable for commerical feeding, because the percentage of protein is about twice that in cereal grains.

Esselbaugh et al. (11) showed that pea protein has good nutritional value; but Satinder et al. (12) found that the value varied greatly between some 28 breeding lines studied. All pea varieties studied by McGinnis[1/] supported chick growth; but growth and PER were significantly improved in nearly all cases by supplementary methionine.

Dry field peas are an agriculturally important crop grown largely in the Palouse area of the Pacific Northwest. They contribute several million dollars to the economy of the region. Farming practices would benefit significantly by more extensive wheat-pea rotation in this area. This practice is one of the best known controls for erosion; however, it is restricted by the limited market for peas. Nearly 90% of the peas harvested in the Pacific Northwest are exported to foreign markets.

The role of dry peas as human food could probably be expanded if additional information about the nutritional and functional food-use properties were available. Textured vegetable proteins and protein isolates (primarily derived from soybeans) are finding their way increasingly into U.S. and foreign food supplies. Other vegetable proteins (besides soy) have potential to share this market.

In this study, we investigated the physical dough properties and baking potential of raw and cooked yellow pea flours as additives to fortify wheat flour.

Materials and Methods

Two pea flours (Good and Best variety) furnished by the Dumas Seed Company, Moscow, Idaho, were used. One was a cooked, ground flour with a protein content of 22.3% and the other, a raw, yellow pea flour, with a protein content of 23.5%.

A defatted soy flour manufactured by Archer-Daniels-Midland Company, had a protein content of 46.5%. It is soy flour routinely used in U.S. Government fortified flour.

The control wheat flour (C-STD) was a commercially milled wheat flour with a protein content of 11.2%, excellent loaf volume potential, and a medium mixing time of about four minutes.

Mixing time, water absorption, and oxidation ($KBrO_3$) or a combination of $KBrO_3$:ascorbic acid were optimized in a 70 min, sugar-free formula (13). No

[1]James McGinnis, Department of Animal Science, College of Agriculture Research Center, Washington State University, Pullman; Personal communication.

nonfat dry milk was used in the formula. In the pea- and soy-fortified breads, pea or soy replaced equal portions of wheat flour to give a total of 100 g (14% mb). Loaf volumes are averages of at least two determinations. Loaf volume differences of 20 cc are significant at P=0.05.

Results

Each blend was baked over an oxidation series with a combination of $KBrO_3$ and ascorbic acid at 75 ppm. Figure 1 shows the loaf volume response of the cooked-pea powder to oxidation. The raw pea and soy flour were similar.

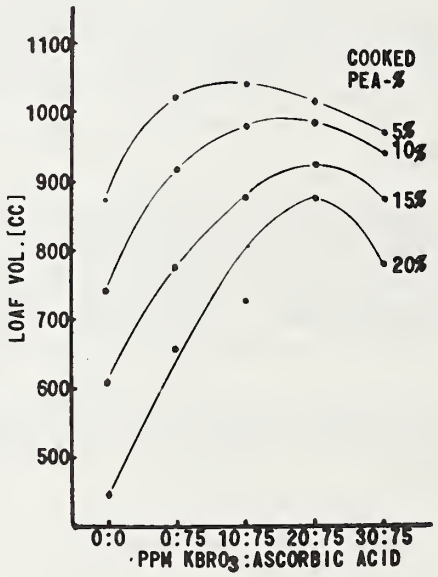

Figure 1. Loaf volume response of cooked pea flour blends to oxidation (75 ppm ascorbic acid with 0-30 ppm $KBrO_3$).

Generally, for each 5% increase in pea flour or soy flour, $KBrO_3$ requirement rose 5 ppm. Baking absorption decreased with increasing levels of raw pea flour (approximately 1.8% decrease/5% supplement increase); however, the cooked pea flour showed an increased water absorption (1.2% increase/5% supplement increase). Baking absorption increased 2.2% for each 5% increase in soy flour.

Mixing time decreased about 3/4 min from the control flour for the first increment of cooked and raw pea flour doughs, but remained nearly stable for each additional level of pea flour. Soy-wheat flour doughs showed increasing mix times with decreased supplementation. These observations in rheological changes can best be shown by mixograms (Figure 2) on the following page.

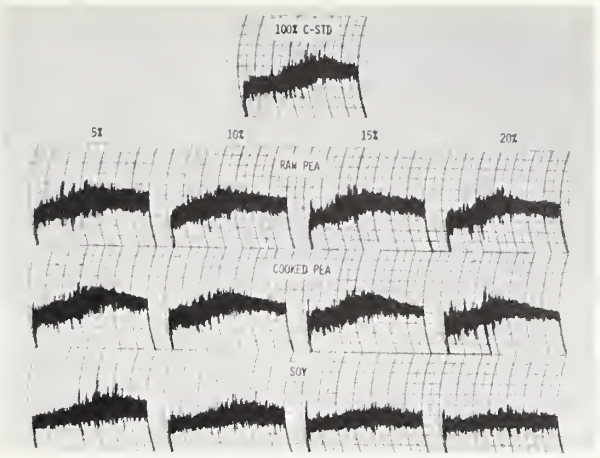

Figure 2. Mixograms of flour blends with raw yellow pea, cooked yellow pea, and soy (ADM's ARDEX 550).

Proof height and time were optimized for each level of pea or soy flour added. Longer proof times were required with increasing pea and/or soy flour additive, resulting in generally higher proof heights to make optimum bread.

Loaf volume decreased with increasing levels of raw pea flour, cooked pea flours, and soy flour (Figure 3). Crumb grain and texture generally decreased

Figure 3. Bread loaves at 5, 10, 15, and 20% replacement with cooked yellow pea flour and soy (ARDEX 550) at various oxidation levels (75 ppm ascorbic plus 0-30 ppm $KBrO_3$.

with increasing levels of the pea flour and soy flour. However, very near standard quality breads were produced when replacing 15% wheat flour by each of the pea flours. It appears that the raw pea flour is slightly more bread functional than the cooked pea flour. The soy-wheat flour bread volume showed a much greater decrease in loaf volume and lower crumb grain scores; thus, it was significantly less functional than the pea flours. Figure 4 graphically summarizes loaf volume responses for the soy and pea flour breads.

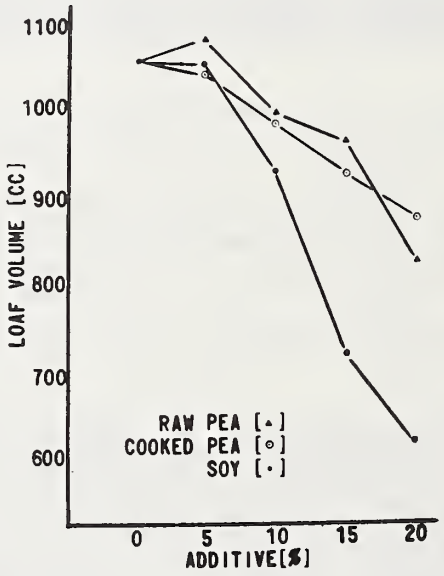

Figure 4. The effects of the pea(s) and soy flours on loaf volume at optimum oxidation levels.

Bread baking studies in Canada using 5, 10, and 15% pea concentrate (air-classified) corroborates our findings that yellow pea flour is highly bread functional[1]. In their studies, 5% vital gluten was added in the formulae. Loaf volume decreased with increased pea concentrate and essentially paralleled our results. Water absorption increased about 1.5% per 5% pea concentrate added. Texture, crumb grain, color, and crust scores decreased with increased pea levels, but 10-15% replacement appeared highly suitable. Pea concentrate had a protein content of nearly 55% (N x 6.25).

A white crumb color was noted with 5 and 10% raw pea flour equal to the control. At higher additive levels, a slight creamy color was observed. At all levels, cooked pea imparted more color. D'Appolonia (9) noted the same results with various legume flour and concluded that perhaps a lipoxygenase-type enzyme in the legume flour was responsible for the improving effect on

[1]Pro-Star Mills Ltd. 817-48th Street East, Saskatoon, Saskatchewan, Canada. Personal communication.

crumb color. We have also baked green pea and find a white crumb grain up to about 15%.

Taste Panel Evaluation

A taste panel was designed as a randomized triangle test in which one-half of the test contained 2 controls with 1 treatment while the other one-half contained 2 treatments with 1 control.

The panel contained 46 persons. Each panelist tasted 4 separate triangle tests. In each triangle test, each individual sample came from the same section of its respective loaf. The first two tests were 5 and 15% raw yellow pea flour respectively, while test 3 and 4 were 5 and 15% cooked yellow pea flour. The control loaf contained 100% wheat flour. The panelists tasted the bread under colored lights so the slight color differences of the 15% level loaves could not be detected.

The panelists were asked to pick out the off sample in each triangle test and to tell why they felt it was different, i.e., (flavor, aroma, texture, etc.). In all cases, (Table I) more panelists picked out the 15% levels than the 5% levels, but in every case, the results were not significant.

TABLE I. Triangle taste panel evaluation of 5 and 15% of both cooked and uncooked (raw) pea flour breads.

	TASTE PANEL			
	RAW PEA 5%	RAW PEA 15%	COOKED PEA 5%	COOKED PEA 15%
Number of Panelists Correct in Selecting Odd Sample 1/	17	18	9	19
Number of Panelists Incorrect in Selecting Odd Sample	29	28	37	27
TOTAL	46	46	46	46

1/ Twenty-two correct responses were needed to be significant at the 5% level using 46 panelists.

Pea, Soybean, and Wheat Protein Components

The data in Table II shows a comparison of protein and the essential amino acids for human nutrition for pea, soybean, and wheat flour as reported by FAO (14). Protein content of the pea is slightly more than one-half that of the soybean. Significant differences appear to exist in the essential amino acids leucine, lysine, methionine, and possibly phenyaline.

TABLE II. Protein and some selected amino acids of soybean, pea, and wheat flour.[1/]

PROTEIN AND ESSENTIAL AMINO-ACIDS[1]

	Pea (Pisum sativum)	Soybean (Glycine max)	Wheat Flour 70-80% Extraction
Moisture (g/100g)	11.00	8.00	12.00
Nitrogen (g/100g)	3.60	6.65	1.91
Conver. factor(N)	6.25	5.71	5.70
Protein (g/100g)	22.50	38.00	10.90

AMINO ACIDS ESSENTIAL FOR HUMAN NUTRITION

	mg/g Total Nitrogen		
Isoleucine	267	284	228
Leucine	425	486	440
Lysine	470	399	130
Methionine	57	79	91
Phenylalanine	287	309	304
Threonine	254	241	168
Valine	294	300	258

[1/] From FAO, Amino Acid Content of Foods and Biological Data on Proteins No. 24 (1970).

Figure 5 shows the protein and lysine content of wheat flour with increasing levels (5-20%) of either pea or soybean supplement using data from the FAO Table. Twenty percent pea replacement contributes as much protein as 9.0% soy and as much lysine as 12.8% soy.

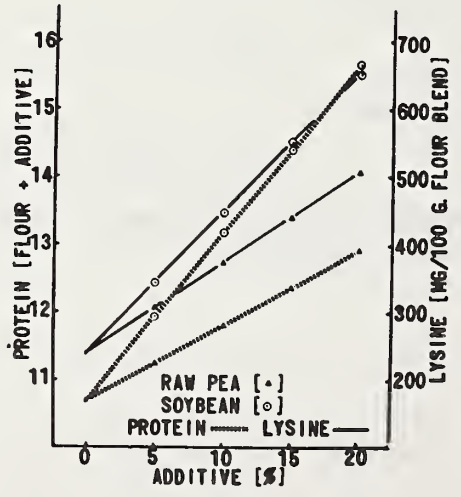

Figure 5. A comparison of protein and lysine levels using pea and soy as additives (replacement) to a common flour (calculated from FAO data in Table II).

Anti-Nutritional Factors

Toxic components such as trypsin inhibitors, hemagglutinins, and phytic acid have been identified in many raw seeds. Table III shows results of analysis of these three components found in soybeans and field peas. Soybeans have considerably higher values of trypsin inhibitors and hemagglutinins than field peas. With these very low levels of anti-nutritional factors in peas, heat processing prior to bread baking would appear unnecessary.

TABLE III. Comparison of the trypsin inhibitor hemagglutinins, and phytic acid levels in pea and soybeans.

ANTI-NUTRITIONAL FACTORS

	Pea (Pisum sativum)	Soybean (Glycine max)
Trypsin Inhibitor (TIU/mg)[1]	2.8	81.0
Hemagglutinins (HU/mg)[1]	9.5	116.0
Phytic Acid (%P)	.70[2]	.49[3]

1) I.E. Liener, U. of Minn. (1977)
2) Kienholz, et al. (1962)
3) Ranhotra, et al. (1974)

Conclusions

This study has shown that certain differences in physical dough and bread baking properties exist among cooked and raw yellow pea and soy flours when blended with a control flour. The pea flours were superior in functional baking performance over the soy at levels above 5%. The soy blends did not make acceptable bread at the 15% level, whereas, cooked and raw pea flour blends produced acceptable breads. No dough improvers (SSL or others) were required to produce acceptable bread fortified with up to 15% yellow pea flour.

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GENETIC RESISTANCE IN CEREAL GRAINS

TO STORAGE INSECTS

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Since the classical studies of Painter (1951, 1958), host plant resistance to insect pests has been the subject of hundreds of publications and dozens of reviews. Some of the most recent and comprehensive reviews were published by Maxwell et al. (1972), Maxwell (1977), and Pathak (1975). According to Painter (1951, 1958), plant resistance to insects can be defined as the relative amount of heritable qualities of a plant, that influence the degree of damage done by the insect. In practice, these qualities allow resistant cultivars to produce larger crops of acceptable quality than are produced by ordinary cultivars exposed to the same level of insect population. Painter divided plant resistance mechanisms into 3 main categories: (a) preference vs. nonpreference; in this mode the plant has an adverse effect on the behavior of the insects, (b) antibiosis; in this mode, the plant has an adverse influence on the growth and survival of the insect, and (c) tolerance; in this mode, a resistant plant supports an insect population without loss of plant vigor or crop yield.

RESISTANCE IN PLANTS

According to Maxwell (1977), considerable progress has been achieved in developing resistant varieties of field crops. The excellent progress in imparting resistance to Hessian fly into wheat has drastically reduced actual losses estimated in the millions of dollars. Moreover, this progress was achieved almost entirely on the basis of selection for resistance, that is, without knowledge of the chemical (or physical) basis of the resistance. There have been many reasons for this situation (Maxwell, 1977). However, to tackle the problem adequately, one must employ a host-plant resistance team that includes plant breeders, plant physiologists, biochemists, analytical chemists, and entomologists. Secondly, resistance to insects in a growing plant involves complex and dynamic interrelationships among the insects, the plants, and the environment, but little is known about the basic behavior and the physiology of the insect, especially as they relate to response to plant components. Finally, until recently, there has been little pressure (or "incentive") to replace traditional, effective, and time-proven chemical insecticides by indefinite and speculative alternatives that would require many years of research.

A major difficulty is, of course, the effect of breeding for resistance to insects on the agrotechnical characteristics of the plant in general and on yield, in particular. As Whitehouse (1970) noted, in a free society economic forces largely determine which species and varieties of plants are grown. If grain with a special chemical composition or nutritive value of with particular end-use properties is not accepted by the grain industry, it is unlikely that the new varieties will be accepted by the farmer. The reason is that adding new

selection criteria (i.e., resistance to insects) limits the choice of plant breeding material and may result in selections that fall behind in yield. Total yield of grain per unit of land is the most obvious measure of success or failure of any cereal crop.

In recent years there has been interest in determining both the potential of resistant plants and the chemical factors that govern the resistance of such plants to insects. This has been brought about by several factors (Maxwell, 1977): the need to develop alternatives to chemical treatment; the demonstrated success of genetic resistance-alone or as part of integrated pest-management programs; new technologies and methodologies, and new information concerning resistance of plants to insects; interest among scientists to engage in team efforts to develop new and challenging approaches to insect control and support-including-funding-for such investigations; and pressures from regulatory agencies and the general public to develop control measures that do not constitute a hazard to nutrition and health.

RESISTANCE IN STORED GRAINS

Although there have been many studies on the resistance in stored grains to insects, the success in finding evidences of resistance has been rather limited. Most studies have dealt with samples of grain commodities which "happened" to include single samples of various types and cultivars or unusual and exotic selections. There have been few systematic studies that have demonstrated varietal resistance in commercial cultivars grown under various environments.

On one hand, a study of resistance in grains stored in a granary is simpler than the study of resistance in growing crops because we are dealing with a more static stiuation. As a result, the interactions and interrelations among such factors as insect physiology, plant physiology, and environment are easier to control. On the other hand, the growing plant has a much greater variety of potential defense mechanisms than threshed grain. The mechanisms and components that could theoretically govern resistance of grain to stored products insects include: (a) mechanical barriers, i.e., tightly adhering hulls, (b) major differences in content of components that affects nutritive value of cereal grains, i.e., high-amylose starch, (c) major components that significantly modify physical properties, i.e., the high protein content that imparts vitreousness, hardness, or resilience, (d) minor components (preferably concentrated in the outer layers) that have a deterrent or specific insecticidal effect, and (e) any combination of (a)-(d), i.e., silica in hulls (combination of a and d) or texture of high-amylose corn (combination of b and c). We will review here some of the studies, mostly done at laboratories where the authors have worked to demonstrate effectiveness of some of these mechanisms in stored cereal grain.

KERNEL STRUCTURE - GENERAL

The cereal grain is a one-seeded fruit, a caryopsis, that has the fruit coat adherent to the seed. As the fruit ripesn, the pericarp (fruit wall) becomes firmly attached to the wall of the seed proper. The pericarp, seed coats, nucellus, and aleurone cells form the bran. The embryo occupies only a small part of the seed. The bulk of the seed is composed of the endosperm, which constitutes a food reservoir (Pomeranz and MacMasters, 1968 and 1970).

The floral envelopes (modified leaves known as palea and lemma) or chaffy parts, within which the caryopsis develops, persist to maturity in the grass family. If the chaffy structures envelope the caryopsis so closely that they remain attached to it when the grain is threshed (as in rice and most varieties of oats and barley), the grain is "covered". If the caryopsis readily separates from the floral envelopes on threshing, as with common wheats, rye, hullness barleys, and the common varieties of corn, the grain is "naked".

THE HULL

The palea and lemma in rice (Fig. 1) and in barley (Fig. 2) are held together by two hook-like structures. The ability of these structures to hold the palea and lemma together without gaps is probably variety-dependent. Insect infestations have been found to be more common in varieties of rice that have many gaps and separations than in varieties with tight husks (Breese, 1960; Russell, 1968; Cohen and Russell, 1970; McGaughey, 1970). Also, the hull is rich in silica. For example, a line scan (X-rays) recorded in an electron microprobe (Fig. 3) shows that silica is concentrated in the outer lemma of barley. Such high and localized content may give protection against insects.

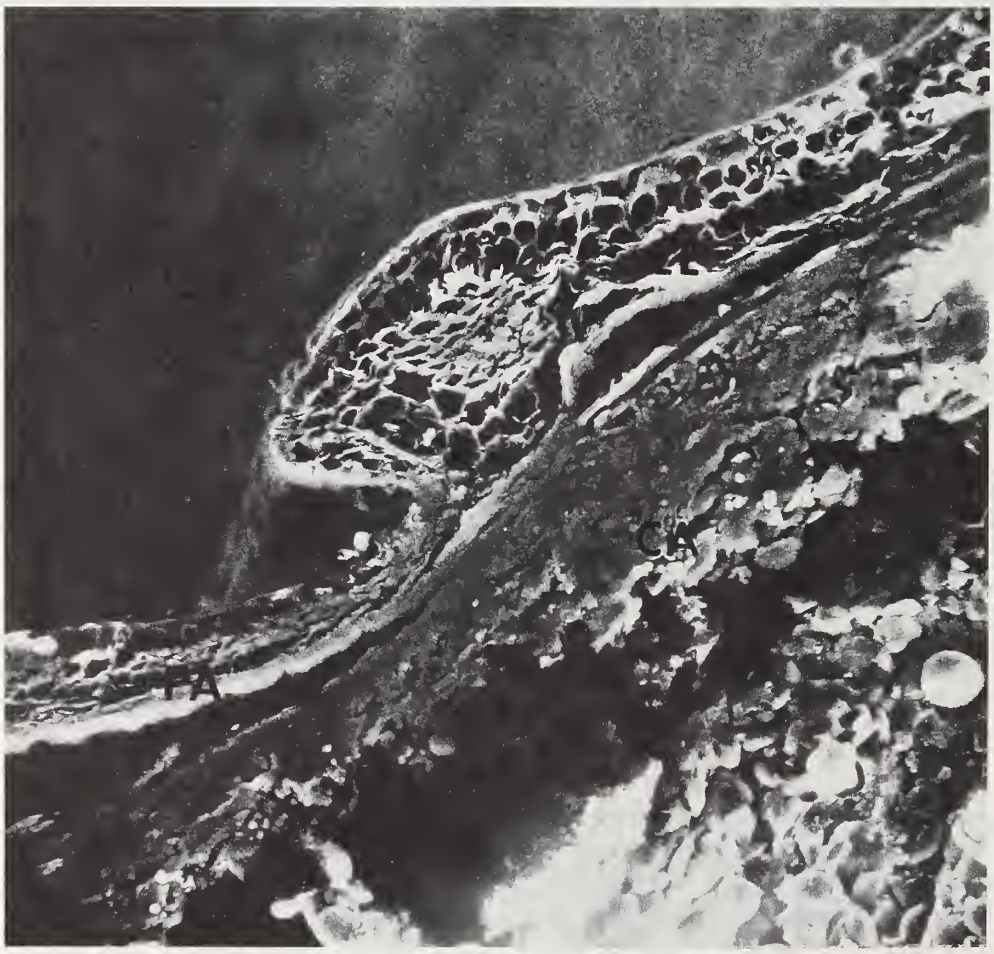

Fig. 1. SEM micrograph transverse section through the palea (PA), lemma (LE), and caryopsis (Ca) of barley. (130 x) (from Pomeranz and Sachs, 1972).

Fig. 2. SEM micrograph transverse section through the palea (PA), lemma (LE), and caryopsis (Ca) of rice (130 x) (from Pomeranz and Bechtel, 1977).

The role of the hull and hull tightness in conferring resistance to the rice weevil, (Sitophilus oryzae, L.) was studied by Boles and Pomeranz (unpublished data) for two naked barley cultivars, Hiproly and CI4362, and three covered barley cultivars, Firlbecks III, Larker, and Conquest. The results are summarized in Tables 1 and 2. An analysis by Duncan's multiple range test indicated large differences in numbers of insect progeny between naked and covered barleys and small differences between barleys with white and blue aleurone layers. There were no significant differences between the six- and two-rowed barleys. Moreover, rice weevils were unable to reproduce in pellets prepared from ground, covered barley grains, which suggested that some component (presumably silica) in the hulls had adverse effects on developing larvae or ovipositing female weevils. Hand dehulled barley grains allowed more rive weevil progeny to develop at a much higher rate and in a shorter time than covered whole grains. However, pellets prepared from dehulled grains allowed about the same level of progeny as covered whole grain. The failure of pellets prepared from covered grains to support insect growth resulted at least in part from the presence of ground hull material.

GRAIN COMPOSITION

In other tests, two naked and three covered barley cultivars, grown at three locations, each with three levels of soil N-fertilization to increase grain protein content (Pomeranz et al., 1976), were infested with rice weevil, and the kernels were examined for progeny starting 35 days after the initial infestation

(Boles and Pomeranz, unpublished data). Neither the cultivar nor the level of soil N-fertilization had a consistent effect on the development time or average weight of the insects. However, average weight loss per insect produced was greater for insects on the covered grain than on the naked barleys, and numbers of rice weevil progenies were higher on the naked than the covered barleys. Among the covered barleys, a blue aleurone cultivar (Conquest) was more resistant to the insects than two white aleurone cultivars (two-rowed Firlbecks III or six-rowed Larker), and the difference was greater when the cultivars had low soil N-fertilization than when they had high soil N-fertilization.

We therefore concluded that some mechanisms (physical barrier effect of the hulls) and some components (protein content) were effective regardless of the environment in which the grain was grown. The significant difference between the blue-aleurone barley and the white-aleurone barleys (Larker and Firlbecks) could be related either to the different physical-textural properties of the two types of grain or to small differences in composition of the aleurone layers only. The latter difference is of greatest interest when one is concerned with developing resistant varieties.

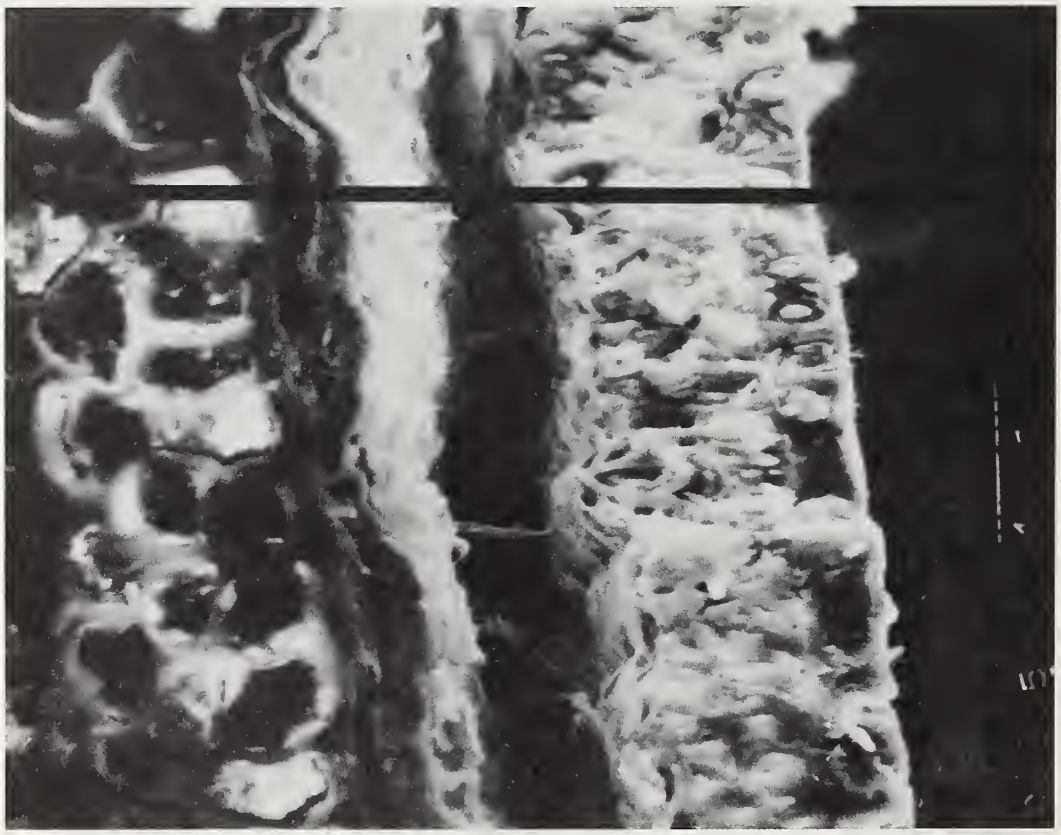

Fig. 3. Line-scan profile for Si across the lemma, pericarp, and aleurone layer (from right to left) of Larker barley; scanned for 250 sec. at 20 V, 2 x 10^{-9}A. Bottom: SEM microphotograph (600 x); top: Si-line scan profile (from Liu and Pomeranz, 1975).

Table 1. Number of rice weevil progeny from grains harvested from five barley cultivars fertilized at different rates during growth. Figures for each cultivar and fertilizer rate are means of three growth locations, each replicated three times, and each replication, in turn, sample-replicated five times.[a]

Cultivar	Hull	Row	Aleurone color	No. Progeny at indicated[b] Fertilizer rates N-0	N-Opt.	N-2x Opt.	Pooled rates
				No.	No.	No.	No.
1. Hiproly	Naked	Two	White	A 34.8[a]	A 30.8[a]	A 38.1[a]	A 34.6
2. CI 4362	Naked	Two	White	B 58.7[a]	B 58.9[a]	B 57.1[a]	B 58.2
3. Larker	Covered	Six	White	A 30.5[a]	A 21.0[a]	C 20.0[a]	C D 23.8
4. Conquest	Covered	Six	Blue	C 17.5[a]	C 17.7[a]	C 21.6[a]	D 18.8
5. Firlbecks	Covered	Two	White	A 30.1[a]	A C 24.4[a]	C 24.7[a]	C 26.4
1 and 2	Naked	---	---	A 46.8[a]	A 46.6[a]	A 48.8[a]	A 47.4
3,4, and 5	Covered	---	---	B 26.0[a]	B 21.1[a]	B 22.1[a]	B 23.1
3 and 4	Covered	Six	---	A 24.0[a]	A 19.4[a]	A 20.8[a]	A 21.4
5	Covered	Two	---	A 30.1[a]	A 24.4[a]	A 24.7[a]	A 26.4
3 and 5	Covered	---	White	A 30.3[a]	A 22.7[b]	A 22.3[b]	A 25.1
4	Covered	---	Blue	B 17.5[a]	A 17.7[b]	A 21.6[b]	B 18.8
Pooled all cultivars	---	---	---	34.3[a]	30.6[a]	32.3[a]	---

[a] Except for Hiproly which was from two locations only (from Boles and Pomeranz, unpublished data).

[b] Means followed by the same letter do not differ significantly at the 5% level (Duncan's multiple range test).

Table 2. Number of rice weevil progeny from Larker variety covered whole grains, dehulled whole grains, and pellets made from both. Figures are means of three replications with each replication, in turn, sample replicated five times.

	No. Progeny at indicated Fertilizer Rates		
	N-0	Opt.	2XOpt.
Covered Whole Grains	15.0	12.3	7.0
Dehulled Grains	34.0	31.6	26.6
Pellets from Ground Whole Grains	0.0	0.0	0.0
Pellets from Ground Dehulled Grains	16.3	11.3	13.3

(from Boles and Pomeranz)

GRAIN PROCESSING

In still another test, we examined the effect on the oviposition and development of S. oryzae when barley was steeped, germinated and kilned in the laboratory (Boles and Pomeranz, 1977). Parboiled rice, that rice that has been soaked, steamed, cooked, and dried while in the husk and then milled to produce white rice, is modified by the process so the grain has improved nutritional, cooking, and storage attributes. These compositional changes are accompanied by reduced chalkiness and increased vitreousness and translucency. Also, parboiling strengthens the attachment of the germ to the starchy endosperm and prevents separation of the germ during husking. In fact, milling of the husked grain from parboiled rice is more difficult because of the hardening of the endosperm. McGaughey (1974) reported that parboiled milled rice was less suitable than raw milled rice for production of rice weevil as well as for production of lesser grain borer, and five other stored-product insects.

Likewise, in studies with the maize weevil, Sitophilus zeamais Motschulsky, and the lesser grain borer, Rhyzopertha dominica (F.), on bulgar (a gelatinized wheat product), Robinson and Mills (1971) found that both insects developed more slowly in bulgar, especially at high humidities, than in nonprocessed wheat at similar humidities. Boles and Ernst (1972, 1973) demonstrated that WURLD Wheat, another gelatinized product, would not support a population of the red flour beetle, Tribolium castaneium (Herbst) and was not very attractive to the rice

Table 3. Development of rice weevil on steeped, malted, and kilned Larker barley.[a/]

Treatment	Moisture %	Protein %	Mean wt of 100-kernel samples mg	Mean no. progeny produced[b/]	Mean no. development days[b/]	Grain wt loss per insect produced mg	Mean wt 1st 20 insects produced mg
1. No treatment	11.0	12.0	3695	28.2a	41.7a	14.18	1.93
2. Steeped, not malted	12.0	12.7	3572	29.1a	42.7a	13.44	1.76
3. Steeped, high kiln	10.0	12.8	3426	13.1d	48.2bc	17.71	1.68
4. Malted, freeze dried	10.7	12.3	3426	25.3a	47.2b	15.69	1.80
5. Malted, low kiln	10.7	12.5	3553	28.3a	47.9bc	17.36	1.90
6. Malted, high kiln	10.6	12.5	3699	23.0ab	48.9bc	20.26	1.93
7. Malted gibberellic acid 2ppm, low kiln	10.9	12.3	3538	24.9ab	48.0bc	17.13	1.81
8. Malted gibberellic acid 2ppm, $KBrO_3$ 800 ppm, low kiln	10.5	12.6	3922	15.9cd	46.8b	17.10	1.99
9. Commercial malt	10.8	12.8	3596	19.0bc	49.1bc	16.92	1.75

[a/] Means are for 2 tests, each with 5 replicated 100-kernel samples for each treatment; each sample infested for the oviposition period with 6 female and 3 male weevils.

[b/] From start of oviposition to emergence of progeny.
Means followed by the same letter do not differ significantly at the 5% level (Duncan's multiple range test).

weevil, Sitophilus oryzae; also, developmental periods were longer and fewer progeny emerged on processed than on unprocessed wheats. Mills (1973) had similar results when he tested WURLD wheat against the maize weevil.

The research we (Boles and Pomeranz, 1977) obtained with barley was similar (Table 3). The oviposition and growth of Sitophilus oryzae were reduced by high kilning of malt, especially of barley, but the addition of gibberellic acid (to enhance enzyme synthesis) had no such effect. However, gibberellic acid plus potassium bromate added to stop proteolytic activity inexplicably reduced numbers of progeny. The main effects of barley processing on rice weevil development were from the heat treatment.

WHAT ABOUT THE FUTURE

Any survey of studies of inherent differences in resistance of stored grains to insects shows that few systematic studies have been conducted and that those unlikely that many resistant traits will be discovered in the near future. Also, it is even less likely that any such traits that are found can be incorporated easily into existing and established cultivars. Mechanisms such as high protein content and the vitreousness associated with it are available but can only be incorporated in certain types of grain, i.e., durum and high-protein bread wheats. Indeed, a high amylose content makes cereal grains nutritionally unavailable to both insects and mammals. Also, the likelihood that a single compound present on the surface (or outside layers) of grain will be toxic enough to control infestation is small, but if a combination of compounds is required, breeding problems would be greatly compounded.

Nevertheless, there are indications that breeding for resistance to stored-grain insects is possible. It may be necessary to find a reasonable compromise between such a resistance and maximum yield. In addition, any inherent resistance will probably not give complete insect control though it could be used to advantage as part of an integrated pest control program. For many years, plant breeders have looked at desirable agrotechnical characteristics (mainly yield) and have overlooked undesirable nutritional and storage characteristics. We are reaching a point where yield may not be all important. Instead, we may try to produce more nutritious grain that will store better and longer.

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DIELECTRIC PROPERTIES OF WHEAT AND POSSIBILITIES FOR CONTROL OF STORED-GRAIN INSECTS BY DIELECTRIC HEATING

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Electrical properties of grain are of interest for two principal reasons. First, the electrical properties of grain are highly correlated with its moisture content, thus providing a convenient means of rapidly estimating moisture content. Second, the electrical properties, specifically the dielectric properties, determine the behavior of grain when it is subjected to electromagnetic fields in such processes as radiofrequency or microwave dielectric heating. The dielectric heating applications have been considered for use in accelerating grain drying and in controlling stored-grain insects. Neither of these applications has yet been accepted for practical application, however, mainly for economic reasons. On the other hand, electrical moisture meters have long been used for practical measurement of grain moisture content.

The purpose of this paper is to summarize data on the dielectric properties of wheat and their dependence on the moisture content of the wheat and on the frequency of the applied electromagnetic field and to describe how this kind of information is applicable to the problem of controlling stored-grain insects by radiofrequency (RF) dielectric heating. Some additional comments are included on the potential for practical application of RF insect control methods.

Basic Principles. Dielectrics are a class of materials usually considered to be relatively poor conductors of electricity. The dielectric properties of usual interest are the dielectric constant, ε_r', the dielectric loss factor, ε_r'', respectively the real and imaginary parts of the complex relative permittivity, $\varepsilon_r^* = \varepsilon_r' - j\varepsilon_r''$, the loss tangent, $\tan \delta = \varepsilon_r''/\varepsilon_r'$, and the conductivity, $\sigma = \omega\varepsilon_o\varepsilon_r''$, where δ is the loss angle of the dielectric, ω is the angular frequency of the applied field ($2\pi f$, where f is the frequency), and ε_o is the permittivity of free space (8.854×10^{-12} farad/m). These properties have been defined in greater detail, both in terms of electrical circuit concepts (1) and electromagnetic field concepts (2). Basically, the dielectric constant of a material, ε_r', is related to the capability of that material for storing energy in an electric field in the material, whereas the loss factor, ε_r'', is related to the material's capability of absorbing energy from the field. The dielectric constant of a material is frequently considered as the ratio of the capacitance of a capacitor, with the material as its dielectric, to the capacitance of the same capacitor with air or vacuum as its dielectric. The loss factor is an index of the energy dissipation characteristics of the material when it is exposed to RF electric fields.

The loss-tangent value is also indicative of a material's energy dissipation characteristics. The conductivity, which may be expressed as $\sigma = 0.556\ f\ \varepsilon_r''$ mho-cm^{-1}, is the reciprocal of the specific volume resistivity, which is the resistance between opposite faces of a centimeter cube of the material.

All of these dielectric properties are frequency dependent, as well as temperature dependent, for most materials that may be considered as dielectrics. Biological materials such as grain and insects have dielectric properties that are dependent upon frequency. This frequency dependence was the basis for a study of the dielectric properties of insects and grain to determine the optimum frequency range for control of the insects by RF dielectric heating (3).

The heating of dielectric materials in a radiofrequency electric field results when electric energy is absorbed from the field and converted to heat energy in the material. The rate of energy dissipation, or power dissipated in the material, can be expressed as $P = 55.63\ f\ E^2\ \varepsilon_r''$ watts/m^3, when f is in megahertz (MHz) and E is the rms value of the electric field intensity in kilovolts/m. The heating rate of the material will depend upon both its specific heat and the power dissipation. However, the power dissipation in different components of a mixture of materials, e.g., insects in grain, will depend upon the relative values of the dielectric properties of the components of the mixture. The power dissipation depends directly upon the dielectric loss factor and indirectly upon the dielectric constant, because the field-intensity distribution in the mixture is a function of the relative values of the dielectric constants and geometric factors. Thus, if the frequency dependence of the dielectric properties of the components of the mixture differs or if the relative values of the properties of the components are sufficiently different, selective heating of one component of the mixture by dielectric heating may be possible if the proper frequency range is used.

Dielectric Properties of Wheat. The frequency and moisture dependence of the dielectric properties of hard red winter wheat have been studied over a frequency range from 250 Hz to 12.1 GHz at moisture contents ranging from 2.7 to 23.8 percent moisture, wet basis (4). Averages of measured values for the dielectric constant and loss factor of lots of seven varieties of hard red winter wheat are shown in Figs. 1 and 2. These curves illustrate the range in values of ε_r' and ε_r'' and their variation with frequency, but because of the logarithmic scales necessary to display data over such wide ranges of frequency and moisture content, they do not illustrate dielectric property-vs-moisture relationships very well. Moisture dependence of ε_r' and ε_r'' in different frequency ranges is better illustrated in Figs. 3 and 4.

Behavior of the dielectric constant is regular, with respect to frequency, in that it either decreases or remains constant with increasing frequency. Its behavior with respect to moisture content is also regular

in that it either remains constant or increases with increasing moisture content. The dielectric loss factor is regular with respect to moisture content in the moisture range studied, but it may either increase or decrease with increasing frequency depending upon the moisture-content and frequency range of interest. At low moisture contents, there is evidence of a broad region of dielectric dispersion and absorption centered at about 10 MHz (2,4).

Stored-Grain Insect Control. The broad dielectric absorption region at about 10 MHz was also observed for wheat in measurements to determine the best frequency range for stored-grain insect control by selective dielectric heating (3). A much more prominent dispersion and absorption was found in the dielectric properties of adult rice weevils (3). Results of measurements on hard red winter wheat and adult rice weevils are summarized in Figs. 5 and 6. Upon taking into account the factors that influence the power dissipation in a mixture of dielectric materials, the frequency region between 10 and 100 MHz was identified as the most promising region for selectively heating the insects (3). Since the insect-to-grain dielectric-loss-factor ratio is the most important factor that affects the differential dielectric heating of the two materials, the advantage of the 10- to 100-MHz frequency range is evident from data in Fig. 6.

Data from the literature on experimental treatment of stored-grain insects at frequencies of 39, 90, and 2,450 MHz tended to support the theoretical predictions for selective dielectric heating based on measured values of the dielectric properties of insects and grain (3). However, a direct comparison was made by exposing adult rice weevils in hard red winter wheat to RF electric fields at frequencies of 39 and 2,450 MHz for varied time intervals. Results of these experiments, which are summarized in Fig. 7, confirmed the predicted superiority of the lower frequency for controlling the insects by selective dielectric heating. Complete mortality of the insects 8 days after treatment was achieved by 39-MHz exposures that heated the wheat to 40° C., whereas, at 2,450 MHz, exposures resulting in wheat temperatures of more than 80° C. were required for complete mortality of the insects.

Practical aspects of radiofrequency energy application for stored-grain insect control have been considered (5, 6, 7). For conditions prevailing in the United States and Canada, RF methods appeared to be more costly than chemical control methods. Recent economic analyses in Switzerland, however, indicated that radiofrequency insect control might be achieved at lower costs than those of chemical control (8). The principal advantage of RF insect control is the lack of potentially harmful chemical residues. If RF insect control were to become practical, however, even better sanitation practices than are presently used would probably be necessary to prevent reinfestation, because no residual protection would be provided.

The potential for future practical application of RF methods for controlling stored-product insects depends mainly upon economic factors and food safety policies. Research has shown that the method can be effective. Practical-scale tests have also been carried out successfully (9). Further improvements in efficiency of the method may be possible (5, 6, 7), but substantial investments in equipment would be required and sizable amounts of energy would be needed. RF methods, however, do constitute a potential alternative should chemical methods become unsatisfactory.

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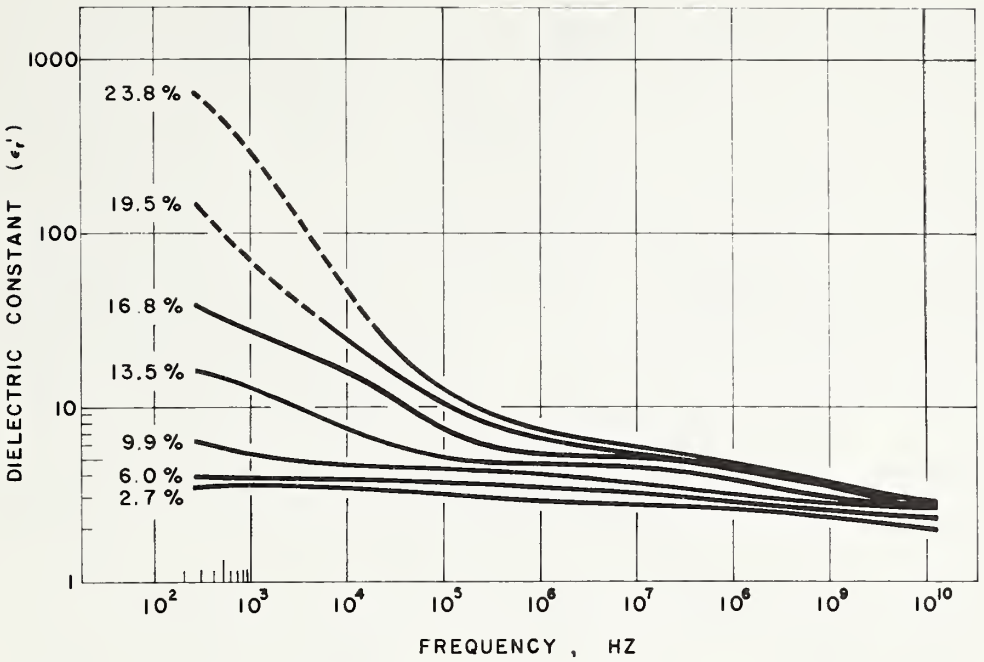

Fig. 1. Frequency dependence of the dielectric constant of hard red winter wheat at 24°C. and indicated moisture contents (4)

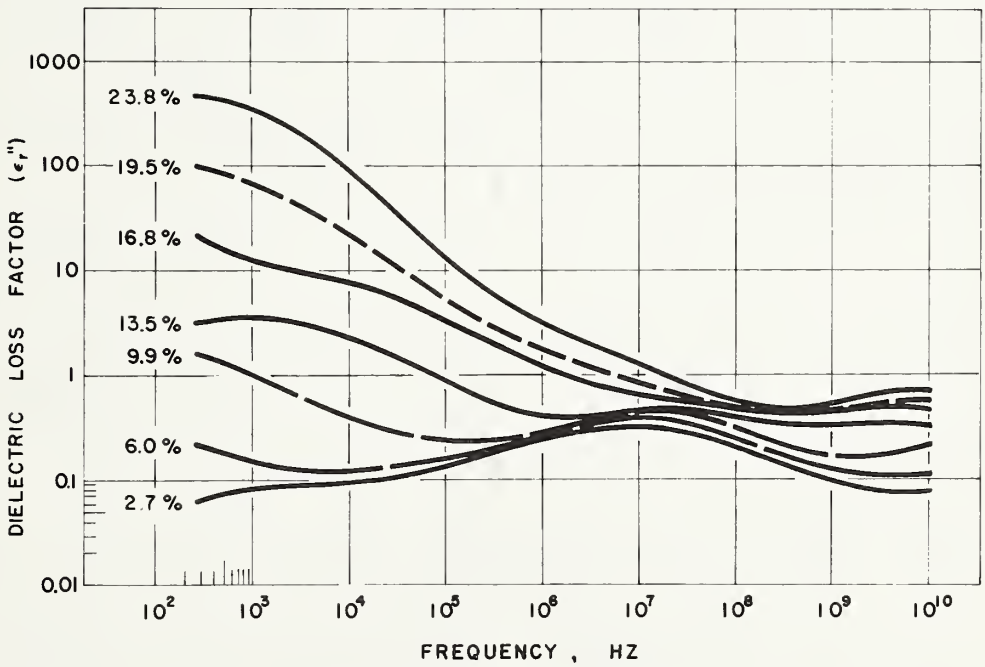

Fig. 2. Frequency dependence of the dielectric loss factor of hard red winter wheat at 24°C. and indicated moisture contents (4)

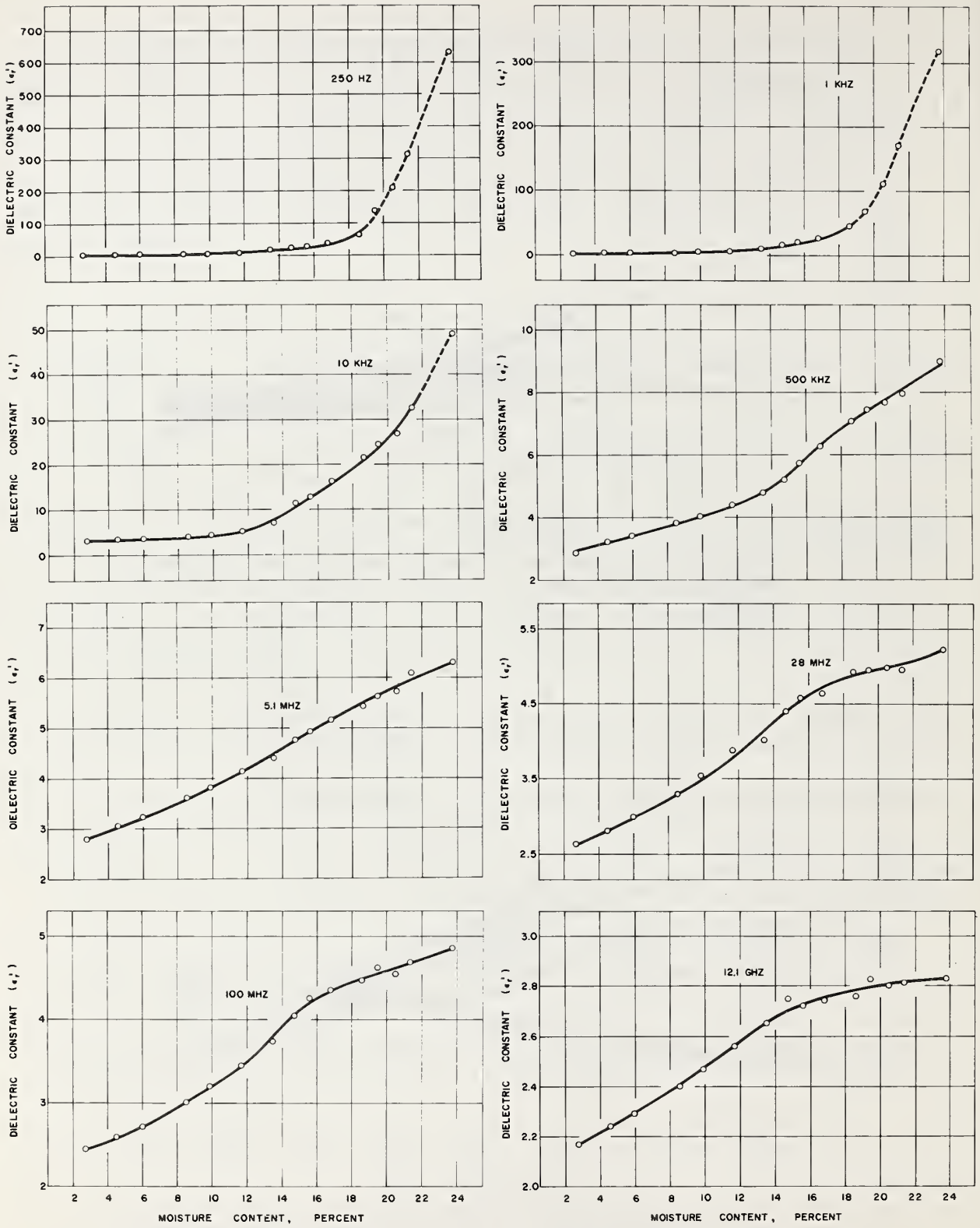

Fig. 3. Moisture dependence of the dielectric constant of hard red winter wheat at 24°C. and indicated frequencies (4)

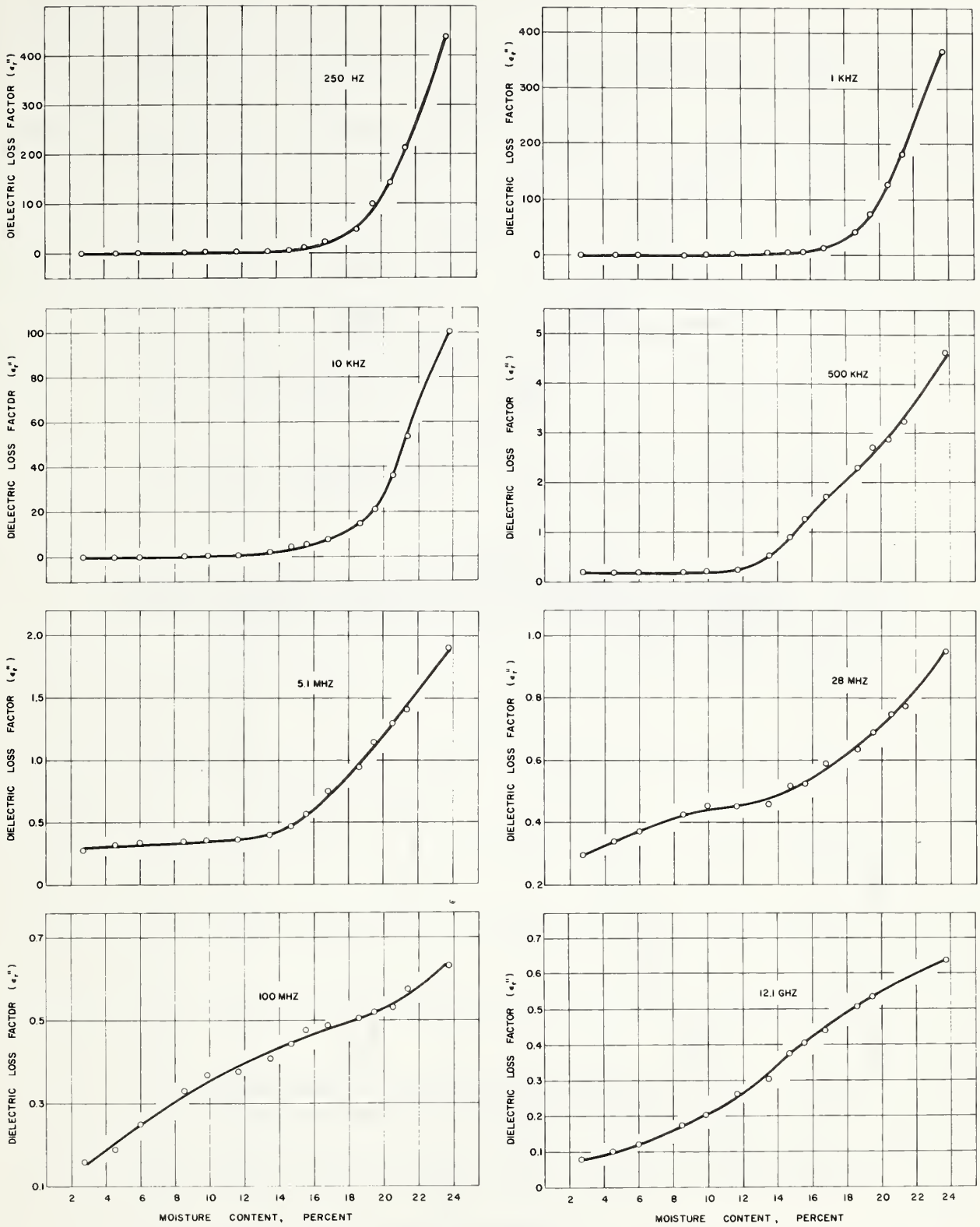

Fig. 4. Moisture dependence of the dielectric loss factor of hard red winter wheat at 24°C. and indicated frequencies (4)

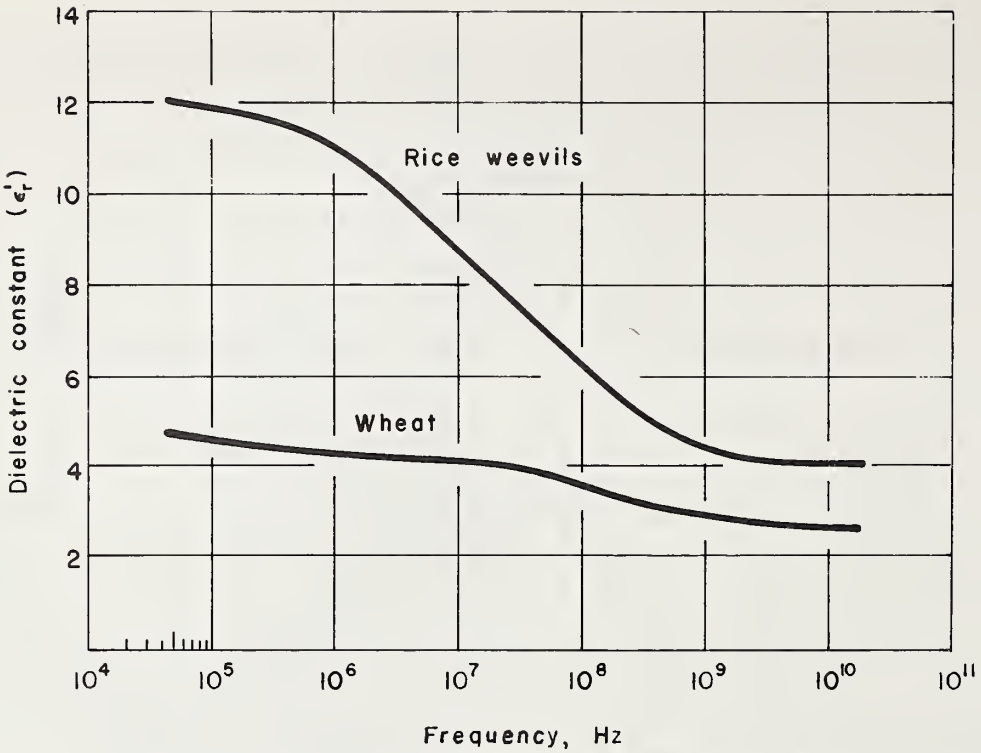

Fig. 5. Dielectric dispersion of bulk samples of adult rice weevils and of hard red winter wheat (10.6% moisture) at 24°C. (6,7)

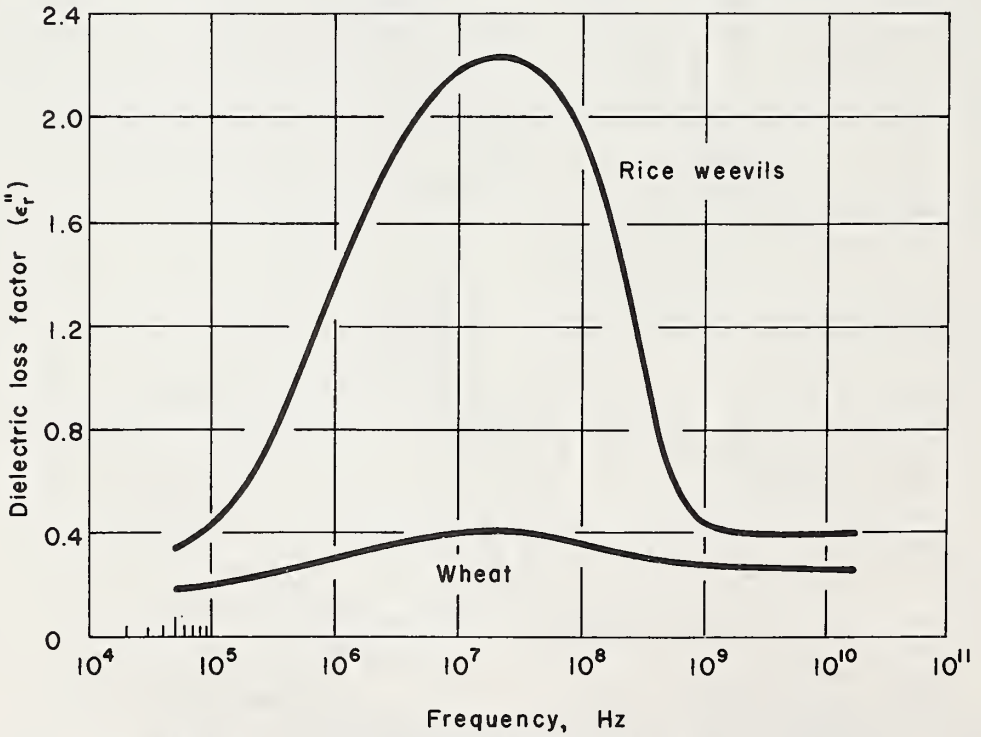

Fig. 6. Dielectric absorption of bulk samples of adult rice weevils and of hard red winter wheat at 24°C. (6,7)

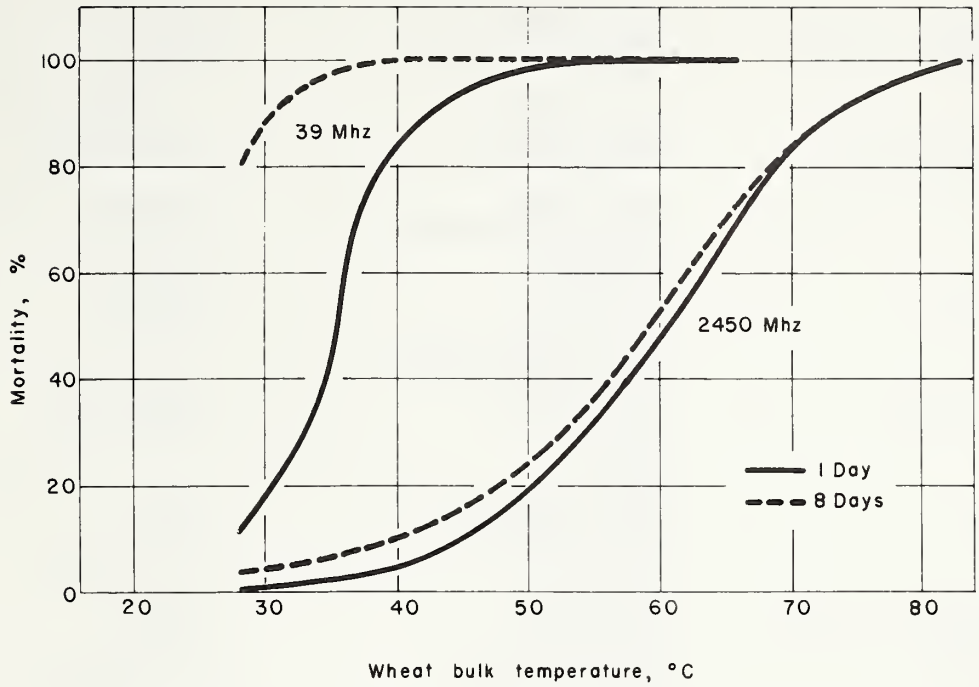

Fig. 7. Comparison of mortalities of adult rice weevils treated in hard red winter wheat at frequencies of 39 and 2,450 MHz (mortalities observed 1 and 8 days after treatment) (7)

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